Plant Pathology & Quarantine 9(1): 30–42 (2018) ISSN 2229-2217
www.ppqjournal.org **Article**

Doi 10.5943/ppq/9/1/4

Molecular diversity and distribution of arbuscular mycorrhizal fungi colonizing peach (*Prunus persica*) and apple (*Malus pumila*) trees in a sustainable small market garden

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Winther J, Aldrich K 2019 – Molecular diversity and distribution of arbuscular mycorrhizal fungi colonizing peach (*Prunus persica*) and apple (*Malus pumila*) trees in a sustainable small market garden. Plant Pathology & Quarantine 9(1), 30–42, Doi 10.5943/ppq/9/1/4

Abstract

We characterized the molecular diversity and distribution of arbuscular mycorrhizal fungi (AMF) in the roots of apple and peach trees found at Grand Valley State University's Sustainable Agriculture Project small market garden. Arbuscular mycorrhizal fungal root colonization observed in cleared and stained roots ranged from 11–43%. The molecular identity of the fungal symbionts was determined based on phylogenetic analyses of isolated small subunit ribosomal DNA sequences. Twenty-seven arbuscular mycorrhizal fungal sequences in the phylum Glomeromycota were isolated from roots of apple and peach trees with 96% of those sequences in family Glomeraceae including the genera *Rhizophagus* and *Sclerocystis*, and 4% of the sequences share identity with fungi in the family Paraglomeraceae. Four of the isolated arbuscular mycorrhizal sequences were shared between different apple and peach trees. Peach tree roots had the highest arbuscular mycorrhizal richness of trees sampled. Our analyses suggest that apple and peach trees in small gardens may form arbuscular mycorrhizal associations with different fungi than apple and peach trees of larger scale agricultural operations. Furthermore, the presence of shared AMF sequences between different fruit trees suggests the presence of common mycorrhizal networks that may serve an important function in the health and productivity of small market gardens.

Key words – agriculture – Glomeraceae – mutualism – symbiosis – 18S

Introduction

The most common type of mutualistic mycorrhizal association, found in about 80% of plants, is an arbuscular mycorrhizal (AM) association (Smith & Read 2008). AM fungi (AMF) benefit their plant host by increasing access to limiting mineral nutrients, increasing drought tolerance, increasing pathogen resistance, increasing growth, and facilitating better soil structure (Gnekow & Marschner 1989, Morin et al. 1994, Leake et al. 2004, Cavallazzi et al. 2007, Parniske 2008, Gianinazzi et al. 2010, Smith & Smith 2011, Pozo et al. 2013, Schouteden et al. 2015). In exchange for the increased access to nutrients and associated benefits, the fungal symbiont receives fixed sugars from the plant that are necessary for growth and reproduction (Johnson et al. 1997, Leake et al. 2004, Parniske 2008, Smith & Read 2008). AM associations are particularly valuable in an agricultural setting where increased access to limiting mineral nutrients can increase yield, decrease loss to pests, minimize synthetic fertilizer use, and decrease negative impacts to the environment (Plenchette et al. 1981,

Submitted 9 October 2018, Accepted 8 December 2018, Published 1 March 2019
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1983, Morin et al. 1994, Jeffries & Barea 2001, Jeffries et al. 2003, Cavallazzi et al. 2007, Gianinazzi et al. 2010, Pozo et al. 2013). Thus, the potential for AM fungal associations to increase farming efficiency has led to the research and development of AM inoculums used in both conventional and sustainable agriculture practices (reviewed in Ijdo et al. 2011, Rouphael et al. 2015). However, recent studies have demonstrated that not all AM fungal partners are equally beneficial to the host plant (Van Der Heijden et al. 1998, 2003, 2015, Klironomos 2003, Koch et al. 2006, Jansa et al. 2008, Maltz & Treseder 2015, Werner & Kiers 2015). In addition, the identity of AM associates varies based on the agricultural practices (Bainard et al. 2015, Van Geel et al. 2015, Gottshall et al. 2017, Turrini et al. 2017). Therefore, it is critical to know the identity of the AM community in the crop plants and agricultural system of interest as a first step in assessing and managing AM associations.

The fungi that form AM associations are in the phylum Glomeromycota, a group of fungi characterized by an asexual lifecycle, aseptate hyphae, and forming symbiotic AM associations with plants (Schüßler & Walker 2010). Plants with AM associations have intercellular highly branched or coiled hyphal structures (arbuscules), that are the site of mineral and nutrient transfer between the plant host and the fungal symbiont. Intracellular vesicles (terminal bulbous hyphal structures used for fungal nutrient storage) are a common feature of AM associations (Smith & Read 2008). AM associations are typically characterized by the percent of root length colonized by intercellular hyphae and the types of arbuscules and vesicles present (Giovannetti & Mosse 1980, Jeffries et al. 2003, Gorzelak et al. 2012, Treseder 2013, Soudzilovskaia et al. 2015). While morphological characteristics may serve as proxies for the function of AM associations, it is not possible to determine the taxonomic identity of the fungal symbiont based on the morphological characteristics of the root association (Merryweather & Fitter 1998, Morton & Redecker 2001, Jeffries et al. 2003, Gorzelak et al. 2012). To identify the AM fungal symbiont, molecular phylogenetic analyses must be used; analyses of the ribosomal small subunit (18S) being the most common (Schüßler et al. 2001, Jeffries et al. 2003, Schüßler & Walker 2010, Gorzelak et al. 2012). Establishing the identity of the fungal symbiont is fundamental in determining if the same fungal species form associations in all crop plants, and how the identity of the fungus impacts plant health and growth (Morin et al. 1994, Cavallazzi et al. 2007, Parniske 2008). This study focuses on characterizing the molecular diversity of AM fungi present in apple and peach trees on a small sustainable farm using sequence data and morphological investigations of the roots.

Materials & methods

Site

Our research was conducted during the summer of 2014 on fruit trees planted at Grand Valley State University's Sustainable Agriculture Project (SAP). GVSU's SAP is a small market garden farm (<2 acres) dedicated to ecological and economical sustainable farming practices. The soil on the farm from west to east transitions from clay to a clay loam. For over 100 years before 2007, this plot of land was farmed industrially on a soybean/maize rotation with the use of underground drainage tiles that removed water from the clay soil. No pesticides, herbicides, or industrially produced fertilizers have been added to the soil since the complete removal of the drainage tiles in 2008. The fruit trees were planted in 2013 in a single row bordering a no-till field that had been fallow for two years. At the time of our sample collection, trees had been planted for 1 year. The trees were planted about eight feet apart and mulched with wood chips. The nine fruit trees were two Red Delicious apple trees (*Malus pumila* 'Red Delicious'), one Fuji apple tree (*Malus pumila* 'Fuji'), one Cortland apple tree (*Malus pumila* 'Cortland'), one Yellow Delicious apple tree (*Malus pumila* 'Yellow Delicious'), two Redskin peach trees (*Prunus persica* 'Redskin'), one Reliance peach tree *Prunus persica* 'Reliance'), and one Elberta peach tree (*Prunus persica* 'Elberta'). The apples all shared the same rootstock (Malling-Merton 111) and the peach trees shared the same rootstock, Bailey.

Root sample collection

Root samples were collected from each fruit tree two times between 7 May and 18 June 2014 using the JMC 36" soil sampler probe with a ¾ inch diameter and 12-inch long probe. For each collection, three samples were taken from different locations near the base of the tree. Each soil sample core was processed and rinsed with distilled water until only the roots remained. The fruit trees were growing in mulched areas, in the absence of other plants, and all collected roots could be assigned to the fruit tree. Collected roots were divided into samples for sequencing and samples for microscopy. Root samples for sequencing were weighed and divided into 0.1–0.25 g samples and stored at –80°C. Roots designated for microscopy were fixed in formalin-acetic acid-alcohol (FAA) for morphological analysis.

Extraction, amplification, isolation and sequencing of fungal 18S DNA

Fungal DNA was extracted from frozen roots using the DNeasy Plant Mini Kit (Qiagen, USA). DNA extracts from Redskin peach roots underwent the additional steps of ethanol precipitation and gel purification via Qiaquick gel extract kit (Qiagen, USA) before use in PCR. AMF 18S DNA was amplified via polymerase chain reaction (PCR) using GoTaq (Promega, USA) and AMF specific primers AML1-F (ATC AAC TTT CGA TGG TAG GAT AGA) and AML2-R (GAA CCC AAA CAC TTT GGT TTC) (Lee et al. 2008). The PCR program was as follows: 5 minutes at 95 °C, followed by 32 cycles of 95 °C for 30 seconds, 58 °C for 30 seconds, 72 °C for 1 minute, followed by 10 minutes at 72 °C. PCR products were run on a gel and bands that corresponded to the desired size were gel purified with the QIAquick Gel Extraction Kit (Qiagen, USA). These cleaned PCR samples were cloned using the TOPO TA Cloning Kit (Invitrogen, USA). Bacterial colonies were screened for the18S insert via PCR using universal primers M13-forward and M13-reverse. Positive PCR screens were cleaned following the Exo-SAP protocol (Affymetrix, USA) and sent to Annis Water Resource Institute in Muskegon, MI for sequencing. Samples from Redskin peach trees were sent to the University of Arizona Genomics Core for sequencing.

Sequence analysis

Raw sequence data was edited in Geneious (Geneious, Australia). Consensus sequences were generated for sequences that shared ≥ 99% sequence similarity, in all cases the consensus sequence matched a physical sequence. All sequences were blasted in GenBank to confirm the sequences were glomalean through a BlastN search. Sequences have been deposited in GenBank (KX462850–KX462876). Unique sequences were aligned to sequences representing most of the diversity of glomalean sequences (Schüßler & Walker 2010, Redecker et al. 2013) using MUSCLE (Katoh & Standley 2013) (Table 1). Maximum likelihood analyses with bootstrap were conducted using RaxML (Stamatakis 2014) on the CIPRES portal (https://www.phylo.org/). Tree files generated by RaxML were viewed and manipulated in Mesquite (Maddison & Maddison 2018). All sequence alignment files and tree files have been uploaded to TreeBase (http://purl.org/phylo/treebase/phylows/study/TB2:S23062?x-access-code=f1ee24ac2500e1d94a52f4dcc3e5bf05&format=html).

Morphological analysis

Root samples that were fixed in FAA were cleared in a 5% KOH solution at 55 °C for 2–12 hours, acidified in 5% HCl for 10 minutes, and stained with 1% trypan blue. Stained roots were serially infiltrated to 100% EtOH, and then serially infiltrated to 100% Citrosolve Clearing Agent (Fisher Scientific, USA) before being permanently mounted onto slides with Permount Mounting Medium (Fisher Scientific, USA). On average, ten fine roots ~ 2.5 cm in length were mounted on every slide and 2–6 slides per plant were used to calculate percent of root length colonized by AMF. A standard grid method was used to calculate the percent fungal infection of each slide (Morin et al. 1994).

Table 1 Sequence names and accession numbers used in phylogenetic analysis.

Sequence Name	**Accession Number**	**Sequence Name**	**Accession Number**
Redskin peach B 4	KX462862	*Glomus* sp. W3347	AJ301857.1
Glomus manihotis	U36590.1	*Glomus coronatum*	AJ276086.2
Glomus coremioides	AJ249715.1	*Glomus mosseae*	Z14007.1
Endogone pisiformis	NG_017181.1	*Funneliformis mosseae*	FR750227.1
Glomus sp. BR212	U36592.1	*Glomus* sp. WUM3	AJ301864.1
Acaulospora rugosa	Z14005.1	*Glomus verruculosum*	AJ301858.1
Gigaspora rosea	AJ852608	*Glomus caledonium*	Y17653.2
Scutellospora heterogama	U36593.1	*Glomus* sp.	AJ301865.1
Mortierella polycephala	X89436.1	*Glomus geosporum*	AJ245637.2
Redskin peach B 7	KX462852	*Glomus caledonium*	Y17635.3
Redskin peach B 2	KX462875	*Glomus fragilistratum*	AJ276085.2
Redskin peach A 1	KX462873	*Glomus claroideum*	AJ276075.2
Redskin peach A 2	KX462872	*Glomus* sp. W3234	AJ301855.1
Redskin peach B 1	KX462874	*Glomus luteum*	AJ276089.3
Consensus 3	KX462871	*Claroideoglomus claroideum*	KX879058.1
Glomus sinuosum	AJ133706.1	*Glomus etunicatum*	Y17639.2
Redskin peach B 3	KX462869	*Glomus lamellosum*	AJ276087.2
Consensus 1	KX462868	*Glomus claroideum*.2	AJ276080.2
Reliance peach 1	KX462870	*Glomus lamellosum*.2	AJ276087.2
Cortland apple 1	KX462867	*Claroideoglomus* sp. As-2013	Y17652.2
Redskin peach B 8	KX462858	*Glomus* sp. W3349	AJ301856.1
Glomus clarum	AJ276084.2	*Entrophospora colombiana*	AB220170.0
Rhizophagus proliferus	KX879066.1	*Acaulospora spinos*	Z14004.1
Glomus manihotis.2	Y17648.3	*Entrophospora* sp. WV	Z14011.1
Redskin peach A 5	KX462850	*Scutellospora pellucida*	Z14012.1
Fuji apple 1	KX462851	*Scutellospora dipapollosa*	Z14013.1
Glomus vesiculiferum	L20824.1	*Scutellospora castanea*	AF038590.1
Reliance peach 3	KX462856	*Scutellospora cerradensis*	AB041345.1
Redskin peach B 5	KX462857	*Scutellospora projecturata*	AJ242729.1
Redskin peach B 6	KX462855	*Gigaspora gigantea*	EF014362.1
Redskin peach B 9	KX462859	*Gigaspora albida*	Z14009.1
Redskin peach A 4	KX462866	*Archaeospora trappei*	Y17634.3
Redskin peach A 3	KX462863	*Acaulospora trappei*	AJ006800.1
Reliance peach 2	KX462865	*Geosiphon pyriformis*	Y15905.3
Rhizophagus irregularis	HF968834.1	*Ambispora leptoticha*	AB015052.1
Elberta peach 1	KX462861	*Ambispora leptoticha*	AJ006466.1
Redskin peach B 10	KX462860	*Paraglomus occultum*	AJ276081.3
Red Delicious B	KX462853	Redskin peach B 11	KX462876
Rhizophagus fasciculatus	KX879065.1	*Paraglomus brasilianum*	AJ301862.1
Consensus 4	KX462864	*Paraglomus occultum*	NG_017179.1
Consensus 2	KX462854		

Results

Molecular diversity of AMF

Twenty-seven unique AM fungal sequences from 111 sequenced clones (8–19) clones per fruit tree) were isolated from the roots of nine fruit trees. All 27 sequences are different from AMF

previously identified and databased. Four of the 27 sequences are consensus haplotypes and each of those four haplotypes is an exact match to the most common sequence of all the sequences that share greater than 99% sequence similarity (with most sharing >99.5 similarity). Consensus sequence 1 is representative of 12 sequences from Redskin peach, Reliance peach, Cortland apple, and Yellow Delicious apple. Consensus sequence 2 is representative of 59 sequences recovered from all nine fruit trees. Consensus sequence 3 is representative of 4 sequences recovered from two trees (Reliance peach and Cortland apple) and Consensus sequence 4 is representative of 13 sequences recovered from Redskin peach, Reliance peach, and Red Delicious apple (Table 2). A sampling effort curve (Fig. 1) suggests the number of clones analyzed were sufficient to exhaustively isolate the molecular sequence diversity present in all samples given the curves reach a plateau.

Table 2 Distribution of AMF sequences isolated in roots of apple and peach trees. Trees listed in order planted from east to west.

Fruit Tree Identity	**Shared Consensus Sequence**				**Total # of unique sequences**	**Total # of isolated sequences**	**% root colonized by AMF**
	1	**2**	**3**	**4**			
Redskin peach B					11	13	11.65
Yellow Delicious apple					0	2	23.75
Elberta peach					1	2	19.2
Red Delicious apple A					0	1	26.41
Reliance peach					3	7	38.11
Cortland apple					1	4	16.54
Redskin peach A					5	8	18.53
Fuji apple					1	2	12.5
Red Delicious apple B					1	3	42.67

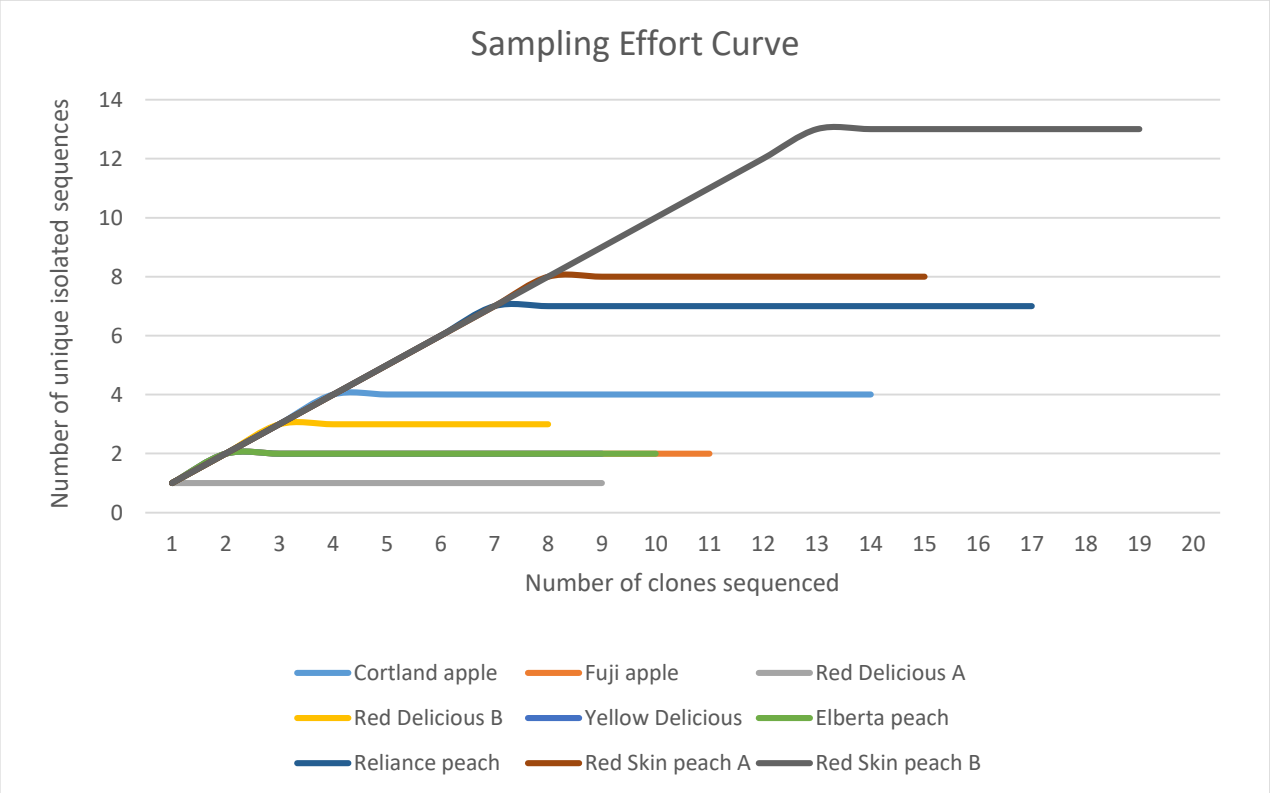

Fig. 1 – Sampling effort curve.

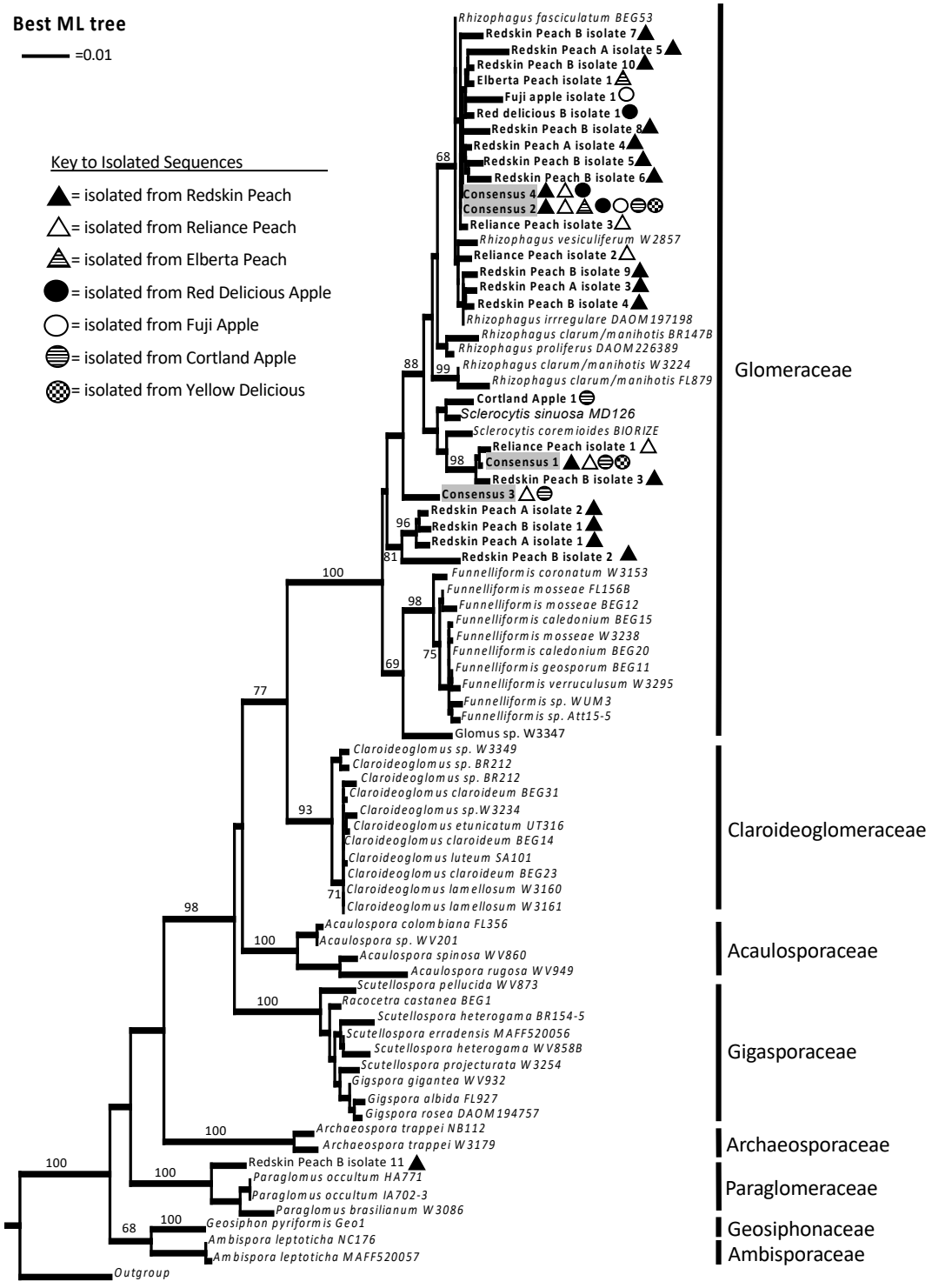

Fig. 2 – Maximum likelihood phylogeny based on 826 characters. Bootstrap values over 65%are shown above the lines.

Our phylogenetic analysis recovered similar topology of Glomeromycota family relationships as reported in Schüßler & Walker (2010), Krüger et al. (2012), Redecker et al. (2013) (Fig. 2). Based on the maximum likelihood phylogenetic tree (Fig. 2), all 27 of the recovered sequences were in phylum Glomeromycota. Twenty-six of the sequences are strongly supported as being in the family Glomeraceae (77 BS) in a clade that includes the genera *Glomus*, *Funneliformis*, *Sclerocystis*, and *Rhizophagus* (100 BS). Seventeen of the Glomeraceae sequences are moderately supported (68 BS)

as being in the genus *Rhizophagus*. Three Glomeraceae sequences are strongly supported (98 BS) as being nested within the genus *Sclerocystis*. The Glomeraceae sequence isolated from Cortland apple roots is strongly supported as being nested within the clade consisting of the genera *Sclerocystis* and *Rhizophagus* (88 BS) even though the relationship to either genera is unresolved. Four Glomeraceae sequences isolated from Redskin peach form a well-supported clade (81 BS) with unresolved relationships to the other defined genera in the family Glomeraceae. Consensus sequence 3 is equally unresolved in terms of its relationship to the Glomeraceae genera *Glomus* and *Funneliformis.* One sequence isolated from Redskin peach is strongly supported (100 BS) as belonging to the family Paraglomeraceae.

Distribution of AMF molecular sequence diversity

The roots of the different fruit trees varied in total number of sequences recovered (1–13) and the number of sequences shared with other fruit trees (1–4) (Table 2). All trees sampled had consensus sequence 2 present. Four of the trees sampled had consensus sequence 1 (Redskin peach trees, Reliance peach, and Red Delicious apple trees). Consensus 3 was recovered from two trees: Redskin peach B and Red Delicious apple B. Consensus 4 was isolated from four trees: Elberta peach, Redskin peach A, Fuji apple and Red Delicious apple B. The number of shared sequences recovered from the fruit tree roots ranged from 1–4. Consensus sequence 2 represented the only shared sequence in 33% of the trees (Elberta peach, Red Delicious apple A, and Fuji apple). Yellow Delicious apple and Redskin peach B had two shared consensus sequences. Reliance peach, Cortland apple and Redskin peach A had three shared consensus sequences; all three trees shared consensus 1 and consensus 2. All four consensus sequences were recovered from Reliance peach.

Twenty-three of the 27 isolated sequences were unique to a single tree, and the number of unique sequences isolated from an individual tree ranged from 0–11. Two of the nine trees had no unique sequences (Yellow Delicious apple and Red Delicious apple A). Elberta peach, Cortland apple, Fuji apple, and Red Delicious apple A had a single sequence unique to that tree. Twenty of the 23 sequences isolated from single trees were recovered from the four peach trees. Redskin peach (A and B) accounted for 70% (16) of the unique sequences isolated in this study, with Redskin peach B having the highest number of unique sequences (11). We calculated Shannon diversity indices (H') using sequences as proxy for species and presence in a particular fruit tree root as proxy for abundance of individuals. For all trees the H'= 2.91, for apple trees H'=1.77 and for peach trees H'=3.07.

Morphological analysis

The percent of AMF infections ranged from 11–43% (Table 2). The greatest percentage of infection was found in the Red Delicious B tree and the least in the Redskin peach B tree. No arbuscules were visible in any of the cleared and stained root samples, but aseptate fungal hyphae and vesicles were observed (Fig. 3).

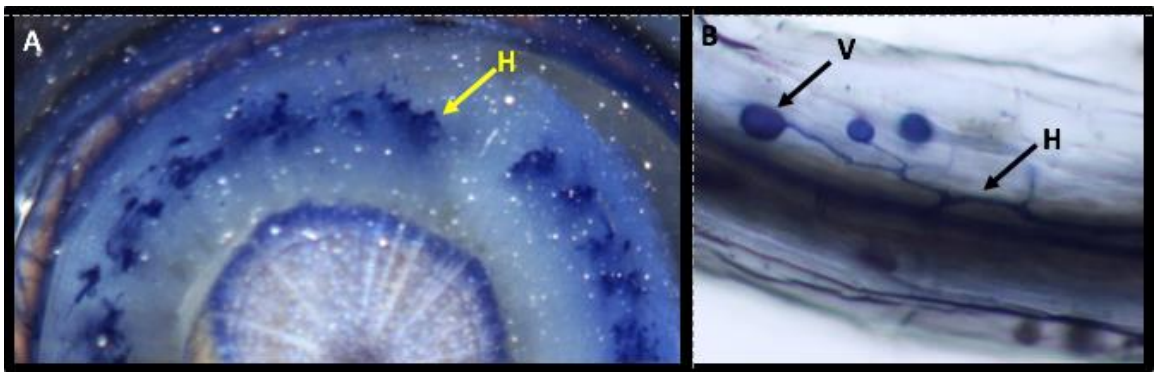

Fig. 3 – Cleared and stained Redskin peach roots. A Cross-section of root. B Later view of root. Hyphae (H), vesicles (V).

Discussion

We isolated and identified 27 DNA sequences from the AMF colonizing peach and apple trees at a small market garden. Of the eight families in Glomeromycota (Redecker et al. 2013), 96% of our sequences belong in the family Glomeraceae and 4% in Paraglomeraceae. Previous studies using molecular techniques to identify AMF associates within apple (Purin et al. 2006, Van Geel et al. 2015, 2016, Turrini et al. 2017) and peach trees (Del Mar Alguacil et al. 2014) have similarly identified most isolated AMF (70–73%) as belonging to the family Glomeraceae. Of the five genera in Glomeraceae (Redecker et al. 2013) we found that 65% of the Glomeraceae sequences most likely belong to the genus *Rhizopaghus.* This is in striking contrast to Turrini et al. (2017) and Del Mar Aguacil et al. (2014) that assigned around 6% of their OTUs isolated to the genus *Rhizophagus.* Both studies identified most of their Glomeraceae sequence diversity as belonging to the genus *Glomus.* Furthermore, we did not recover sequences belonging to Glomeraceae genera *Funneliformis* and *Septoglomus* that were isolated from apples (Turrini et al. 2017) and peaches (Del Mar Aguacil et al. 2014). It is possible that the four sequences recovered from Redskin peach trees and Consensus sequence 3 could belong to the genus *Glomus, Funneliformis* or *Septomglomus* given their position in our phylogeny. Further sampling and morphological work may help resolve their taxonomic identity.

The isolation of AMF in the family Paraglomeraceae from Redskin peach tree roots is consistent with reports of Paraglomeracea in peach trees (Del Mar Alguacil et al. 2014) and apple trees (Van Geel et al. 2016). However, the family Clariodeoglomeraceae is absent from our sampling but comprises 18–26% of AMF sequences identified in previous apple orchard studies in Italy (Turrini et al. 2017) and Belgium (Van Geel et al. 2015, 2016). Moreover, AMF spore surveys in apple orchards in the USA (Miller et al. 1985), Brazil (Cavallazzii et al. 2007), and India (Malik et al. 2016) consistently report the presence AMF in the families Glomeraceae, Claroideoglomeraceae, Acualosporaceae, and Gigosporaceae. The lack of isolates from *Gigaspora* and *Acualospora* from sequence-based studies, including this one, suggest molecular techniques employed may be insufficient for sampling the total diversity of AMF associations in the roots of apple and peach trees.

The AMF diversity we isolated from our apple and peach trees may suggest that the molecular identity of AMF symbionts differs due to a number of factors including age of orchards (1-year old compared to established orchards), tree cultivar/rootstock, agricultural practices (scale and management) and differences due to soil and climate factors. Previous studies have demonstrated that AMF communities are impacted by many factors including climate (Torrecillas et al. 2013), identity of surrounding plants (Van der Heijden et al. 1998, Burrows & Pfleger 2002), age of plants (Montes-Borrego et al. 2014, Herrmann et al. 2016), and agricultural practices (Jansa et al. 2002, Oehl et al. 2004, 2017, Van Geel et al. 2015, Manoharan et al. 2017). More studies identifying fungal symbionts from roots of peach and apple trees in diverse agricultural regions, under varying agricultural practices, are needed to characterize the diversity of AMF found in the roots of apple and peach trees. Greater use of next generation sequencing technology with sequence reads in the range of 600 bp (Shokralla et al. 2014) and employing a variety of primer pairs (Van Geel et al. 2014, Hart et al. 2015) should help resolve AMF molecular diversity across agricultural systems.

Four consensus sequences were shared between peach and apple trees. These shared sequences suggest the presence of extensive shared hyphal networks connecting the trees to one another. The importance of these shared hyphal networks in agricultural settings is largely unknown; however, these common mycelium networks may serve an important role in resource allocation (Van Der Heijden & Horton 2009, Wagg et al. 2015, Walder & Van der Heijden 2015) and coordinating responses to pests (Babikova et al. 2013, Van Der Heijden et al. 2015). In addition, the shared AMF sequences suggest certain AMF are more likely to form associations with diverse crop plants than other AMF species. A meta-analysis of AMF inoculation studies suggests that most AMF inoculants are beneficial to crops and that inoculation with a single AMF species can be the most effective for a particular crop (Van Geel et al. 2016). In small market gardens (characterized by high crop diversity in a small area), it seems best to focus inoculum development efforts on those AMF isolated from many crops, like the AMF isolated in this study represented by consensus sequences 1–4.

The total number of AMF sequences isolated from a tree varied by the type of fruit tree sampled. Overall, peach trees had more AMF richness (as measured by number of sequence isolated, 24) and higher diversity than apple trees. The sequence richness was not spread evenly across the peach cultivars and Redskin peaches had more sequence richness (24) than Reliance (7) or Elberta (2). This suggests that peach cultivars may differ in their symbiotic relationships with AMF, but more research is needed given our small size sample. Apples had a lower Shannon diversity index than peaches (1.77 to 3.07). This is mostly due to the lower AMF richness in apples (1–4) and higher evenness of distribution of sequences between apple cultivars. Given all of our apple trees shared the same rootstock (Malling-Merton 111), the similarity in the number and type of AMF sequences isolated from the different apple cultivars suggests that the rootstock is potentially more important in mediating the AMF community in apple roots than the grafted cultivar. If a goal of managing AMF on a farm includes maintaining a high diversity of AMF (often associated with higher ecosystem function (Van Der Heijden et al. 1998)) and cultivating an AMF community that supplies the greatest benefit to crops, further studies comparing different AMF communities in different rootstocks and with different grafted cultivars is needed. For example, Red Skin peach trees had almost all of the AMF diversity recovered in this study. Therefore, it is possible that planting a single Red Skin peach tree could greatly contribute to maintaining AMF diversity in a small market garden. At the same time, Reliance peach is the only tree sampled that had all four shared AMF. Those four shared AMF isolates may represent AMF generalists capable of forming beneficial associations with the greatest diversity of crops. Therefore, it is possible that planting Reliance peach trees could greatly contribute to maintaining AMF diversity that supply the greatest benefit to the most plants in a small market garden.

In the present study, 11–43% of the apple and peach tree roots were colonized by AMF. These ranges are similar to those reported in apples (Forge et al. 2003, Resendes et al. 2008) and peaches (Wu et al. 2011, Del Mar Alguacil et al. 2014). This suggests that AMF associations in this small market garden are functionally similar to those found in larger scale orchards and potentially providing similar demonstrated benefits such as increased pest resistance, increase growth, and nutrient uptake (Morin et al. 1994, Forge et al. 2003, Del Mar Alguacil et al. 2014, Van Geel et al. 2015). In general, higher infection rates are correlated in many species with increased plant growth (Graham et al. 1991, Treseder 2013) which may suggest that those cultivars with the highest infection rate would eventually have the most benefit from AMF than the other cultivars. Unfortunately, the trees did not survive the 2015 winter and growth/nutrition studies were unable to be conducted. While the percent infection is consistent with previous studies, the lack of arbuscules is puzzling. The presence of vesicles and the lack of arbuscules has been associated with root death, decline of fungal activity and potentially parasitic (less beneficial) nature of an AMF association (Dodd et al. 2000). Resendes et al. (2008) reported that vesicles were mostly present during the months of May and June, when we were collecting. Therefore, the over representation of vesicles could be due to the time of the year. It is also known that different AMF have different infection patterns (Hart et al. 2015). Since the molecular identity of our fungi are different from previous studies, it may be that the AMF infection also demonstrates different characteristics. Clearly, we need to learn more about how the morphology of an AMF association is related to AMF identify, to plant identity, and to functional consequences in agricultural AMF associations in apple and peach trees.

Smaller market gardens (including home and urban gardens) with diverse crops are being promoted as a way to greatly increase sustainable food production (reviewed in Galhena et al. 2013, Eigenbrod & Gruda 2015). Given the small sample size of this study, it is premature to draw any generalizations on the AMF present in apple and peach trees in small market gardens. However, this study documents that most of the molecular diversity of AMF that form associations in peach and apple trees in a sustainable small market garden are in the family Glomeraceae and genus *Rhizophagus*. This molecular diversity is different from that reported from established apple and peach orchards and may represent a real difference based on the scale and/or agricultural practices. Furthermore, the suggested presence of common mycelian networks may help focus the development of AMF inoculum for small scale farms. Moreover, the distribution of AMF between the different

species and cultivars suggests that different trees could have different impacts on the AMF community as a whole. More research is required to understand the AMF diversity in these systems, and how AMF can best be manipulated to maximize benefits in small market gardens.

Acknowledgements

This work was financed by the Summer Student Scholars grant to Winther and Aldrich from Grand Valley State University Office of Undergraduate Research.

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