Project Title: Assessing Barriers to and Benefits of AMF Colonization in Apple

Report Type: Final Project Report

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Budget 1
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Item	2022	2023	2024
Salaries*	34,002.00	34,337.00	34,337.00
Benefits	14,649.00	14,927.00	14,927.00
Wages	NA	NA	NA
Benefits	NA	NA	NA
Sequencing Costs	4,800.00	NA	NA
Lab Supplies	6,595.00	8,088.00	4,736.00
Travel	NA	NA	NA
Miscellaneous	NA	NA	NA
Plot Fees	NA	NA	NA
Total	60,046.00	57,352.00	54,000.00

Footnotes: *GS 11 post-doc, 0.5 FTE

Overall leveraged funding throughout the life of the project: $173,692.00

OBJECTIVES

1. *To characterize the capacity of commercially available arbuscular mycorrhizal fungal (AMF) products and pre-existing AMF communities contained in nursery-derived apple roots to compete with native AMF orchard communities.* Substantially accomplished – see results below. Although we have generally accomplished the goal, in-depth characterization of the sequence data to further explain the results is ongoing).

2. *To identify benefits of specific apple rootstock-AMF associations including protection against pathogenic root fungi, nitrogen uptake, and tolerance to water stress.* Fully accomplished – see results below.

SIGNIFICANT FINDINGS (over the life of the project):

- Plant-AMF relationships are complex and may need to be tailored accordingly. Matching host genetics with compatible AMF species has the potential to enhance agricultural practices in nursery and orchard systems.

- When grown in the presence of replant pathogens (i.e., live orchard soil), G.11 rootstocks pre-colonized with the AMF *R. irregularis* had significantly higher stomatal conductance compared to non-inoculated control plants, regardless of watering regime. This result highlights the role of AMF in maintaining water supply to plants experiencing a combination of water-stress and replant pressure, especially less vigorous apple rootstocks (with relatively small root systems).

- When grown in *live* orchard soil and *well-watered*, G.11 rootstocks pre-colonized by *R. irregularis* recovered significantly more nitrogen (from ^{15}N-enriched fertilizer) than non-inoculated plants growing under the same conditions.

- When grown in *live* orchard soil and *water-stressed* (i.e., combined water-stress and replant pressure), inoculation with *R. irregularis* benefited the ability of G.11 rootstocks to retain/accumulate nitrogen in root tissue. Under these conditions, a plant's need to maintain/prioritize root growth over shoot growth would be expected to be relatively high.

- Results suggest that AMF community composition can be successfully manipulated at the nursery prior or in the field prior to planting. In our experiment, incorporating *R. irregularis* (RTI-Ag) into live soil (at the time of planting) provided several benefits to G.11 rootstocks experiencing replant pressure.

- Spore/propagule count is not directly correlated with the ability of commercially available AMF products to colonize apple roots.

- In general, *Rhizophagus* and *Claroideoglomus* spp. appear to colonize apple root tissue rapidly and maintain strong associations over time. However, product consistency and reliability can be highly variable depending on the manufacturer.

- In G.11, pre-established (nursery-derived) associations with *Rhizophagus* spp. tended to be maintained after planting into both live and pasteurized soil treatments.

- G.11 rootstocks established new relationships with commercially available AMF (previously identified as "promising"), regardless of whether they were planted into pasteurized or live soil (i.e. native microorganisms present in live soil did not prevent these interactions). Rootstocks also established new relationships with a variety of native AMF present in live soil, but associations varied in the presence of commercial inoculum.

- Native microorganisms present in "live" soil can restrict development of and/or alter pre-existing (nursery-derived) AMF communities. Ultimately, however, outcomes between pre-existing AMF communities and biotic components present in soil will depend strongly on the specific groups of microorganisms that are present.

METHODS:

As previously described in the Continuing Report (CR) for 2024, an AMF product assessment was conducted to identify "promising" commercially available products for use in subsequent experiments. **One of these experiments was designed to assess the ability of introduced AMF products to compete with native AMF in live orchard soil and in nursery-derived apple roots (Obj. 1).** Based on product test panel results (CR 2024), *F. mosseae* (RTI-Ag) and *C. claroideum* (RTI-Ag) represented the most "promising" commercially available products in terms of their ability to colonize apple; both products were selected for use in a subsequent greenhouse experiment.

Pasteurization, a proxy for soil fumigation, may provide insight into the ability of commercial AMF products to colonize roots post-fumigation. Therefore, the experiment included both live and pasteurized orchard soil treatments (soil collected from WSU-Sunrise Research Orchard, Rock Island, WA). As described in the original proposal, treatments were: 1) live orchard soil, 2) live orchard soil + *F. mosseae*, 3) live orchard soil + *C. claroideum*, 4) pasteurized orchard soil, 5) pasteurized orchard soil + *F. mosseae*, and 6) pasteurized orchard soil + *C. claroideum*. In addition, G.11 rootstocks from two different nurseries were used: TRECO (Woodburn, OR) and Cameron (Eltopia, WA). Altogether, the experiment included 84 trees (7 replicate trees x 12 treatment/nursery combinations). Prior to planting, a small amount of fine root tissue was collected from various locations on the root system to obtain representative samples of nursery-derived (or pre-existing) AMF communities. This root tissue was stored at −80°C (-112°F) until processing. Trees were planted into 2.7L pots containing live or pasteurized potting mix with or without AMF inoculum (12g per pot). Rootstocks were then grown for a period of 8 weeks under supplemental lighting (16h photoperiod) and watered as needed; plants did not receive supplemental nutrients. Upon harvest, plant growth characteristics including trunk diameter (cm), total plant biomass (g), root volume (mL), and leader shoot length (cm) were measured. At the same time, new (white) root tissue was collected from each plant for DNA isolation/assessment of AMF colonization. DNA extraction (root surface + endosphere) was conducted using the DNeasy Plant Pro Kit (Qiagen, Valencia, CA, USA) according to the manufacturer's instructions. DNA samples were sequenced using Glomeromycota-specific primers (AML1/2) in Fall of 2025. Changes to nursery-derived AMF community composition following

cultivation in orchard replant soil (live vs. pasteurized) were assessed using a Glomeromycota-specific phylogenetic tree, a tool which was previously constructed in collaboration with Dr. Loren Honaas and Dr. Huiting Zhang (as described in CR 2023). This analysis was recently completed (Winter of 2025) and results are reported here.

Assessing benefits of specific apple rootstock-AMF associations (Obj. 2). Experiments designed to explore *functional* benefits of "compatible" apple rootstock/AMF associations were previously conducted as described/reported in CR 2024. One of these experiments, however, could not be completed until Fall/Winter of 2025; the methods and results for this particular experiment are reported here. The primary aim of the experiment was to test for AMF-mediated tolerance to water stress in apple. An additional aim of this study was to assess whether AMF benefits plants in terms of nitrogen uptake. Isotopic labeling of nitrogen represents a powerful addition to the current toolkit with which to analyze the functional benefits of AMF symbioses. Briefly (and as previously described in CR 2024), this experiment was performed using the dwarfing rootstock G.11 because less vigorous apple rootstocks (with relatively small root systems) may become susceptible to water deficits due to the small soil volume exploited by the root system (Casagrande-Biasuz & Kalcsits, 2021). Experimental treatments included both live and pasteurized orchard soil (WSU-Sunrise Research Orchard, Rock Island, WA) with and without the AMF *Rhizophagus irregularis* (formerly *Glomus intraradices*; Mycointech; 12g per 2.7L pot). After 5 weeks, colonization by *R. irregularis* was confirmed via microscopy. Trees were then maintained at 2 different soil moisture contents: well-watered (WW; 80-90% field capacity) and water-stressed (WS; ~40% of field capacity) for an additional 4 weeks. Altogether, there were 8 treatments: Live x No AMF x WW, Live x AMF x WW, Live x No AMF x WS, Live x AMF x WS, Past x No AMF x WW, Past x AMF x WW, Past x No AMF x WS, Past x AMF x WS. These 8 treatments were each replicated 6 times. Volumetric water content sensors were inserted into the root zone (2 per treatment) to ensure target volumetric soil water content was maintained over the duration of the experiment. Stomatal conductance and stem midday water potential (plant physiological responses related to water usage) were measured as described in the original proposal. At the start of the water stress experiment (5-weeks post planting), labelled nitrogen (ammonium-^{15}N nitrate; $^{15}NH_4NO_3$) was spiked into a subset of pots via watering (100 mg per pot). This nitrogen source is physically accessible to both the plants and the AMF. The same amount of unlabeled NH_4NO_3 was added to a different set of pots as a control. Aluminum dishes were placed under each pot to avoid the loss of water containing labelled N and work surfaces were covered with a sheet when using labelled N to avoid cross contamination. After 4 weeks under the different soil moisture regimes, all trees were destructively harvested. Sample processing of leaf, roots, and wood for assessment of labeled nitrogen (^{15}N) uptake were conducted in Fall of 2025 by the UC Davis Stable Isotope Facility (Davis, CA, USA).

RESULTS AND DISCUSSION:

Testing the ability of commercial AMF to compete with those in native orchard soil and those pre-existing in apple roots (i.e., nursery-derived) (Obj. 1): The experiment was harvested in Fall of 2024, microbial DNA present in apple root tissue was extracted, and DNA samples were sequenced. The amount of root tissue colonized by AMF was also assessed at harvest (2 months post-planting). Across treatments, average percent root length colonization ranged between 40-70% in G.11 plants from TRECO and 25-60% in those from Cameron Nursery (as previously reported in CR 2024). No significant differences in percent AMF root length colonization were observed between uninoculated treatments and those containing commercial inoculum (*C. claroideum* or *F. mosseae*), regardless of soil status (live or pasteurized) or nursery origin (TRECO vs. Cameron). However, in the uninoculated TRECO control treatment, pasteurization had a positive effect on the degree of AMF colonization. AMF colonization was significantly higher in TRECO rootstocks grown in pasteurized

soil compared to those grown in the presence of microorganisms contained in live soil (Mann-Whitney test; p=0.04). In comparison, soil pasteurization had no effect on AMF colonization levels in the Cameron rootstocks. This may be partly explained by the fact that initial AMF community composition (prior to planting) was significantly different in G.11 rootstocks from TRECO vs. Cameron nurseries (NMDS; Bray-Curtis dissimilarity index). Taken together, these results suggest that native microorganisms present in live soil can significantly restrict the degree of mycorrhization by pre-established/nursery-derived AMF. However, pre-existing community structure can influence subsequent outcomes between root-associated AMF and soil microbes.

Sequence based analysis of AMF community composition provided more detailed information. Ordination analysis indicated that in the absence of a native soil microbial community (pasteurized soil), inoculation with *C. claroideum* significantly altered pre-established AMF community composition in G.11 rootstocks from both TRECO and Cameron nurseries (1-way ANOSIM; p=0.01 and 0.04, respectively) (Figure 1A and 1B). This result indicates that, in the absence of a functional soil microbial community, *C. claroideum* was competitive and/or antagonistic towards pre-established AMF communities, regardless of differences in starting community structure related to nursery origin. In comparison, inoculation with *F. mosseae*, had no significant effect on the structure of the existing AMF community, regardless of nursery location.

In live soil, AMF community structure varied considerably among treatments. In G.11 rootstocks from TRECO, AMF communities that developed after planting into uninoculated control and *C. claroideum* treatments were significantly different from those of the pre-plant community (1-way ANOSIM; p=0.02 and 0.005, respectively) (Fig. 1C). In G.11 rootstocks from Cameron Nursery, inoculation with *F. mosseae* was the only treatment in which initial AMF community composition was significantly altered (1-way ANOSIM; p=0.004) (Fig. 1D). These results suggest that outcomes between biotic components in the live/bulk soil and pre-existing AMF communities will depend on the specific groups of microorganisms that are present in each compartment.

Phylogenetic analysis was conducted to further assess whether the apple rootstocks served as a significant source of inoculum from the nursery where they were produced**.** In the TRECO rootstocks, the nursery-derived AMF community was represented only by *Rhizophagus* spp. which included a diversity of ASVs (Fig 2, Clade 1). Most of these associations were maintained at relatively high abundance across both pasteurized and live treatments, except when rootstocks were grown in live soil + *C. claroideum* (a treatment which was characterized by the loss of several *Rhizophagus* ASVs).

In comparison, the AMF community pre-established in rootstocks from Cameron nursery was much more diverse, representing ASVs from all 4 main clades (*Rhizophagus*, *Glomus, Funneliformis*, and *Claroideoglomus* spp.) as well as a handful of "unknown" AMF, whose sequences could not be assigned to any known taxonomic group (Fig 2, Clade 6). Unlike TRECO rootstocks, AMF communities pre-established at Cameron nursery included only a single ASV from the *Rhizophagus* group (ASV 3 – also dominant in TRECO rootstocks). However, this ASV was maintained in all pasteurized and live treatments, except when rootstocks were grown in live soil + *F. mosseae.* Regardless of nursery location, associations with *Rhizophagus* that were established prior to planting tended to be maintained across both live and pasteurized treatments.

As mentioned above, no *Glomus* spp. ASVs were detected in TRECO rootstocks prior to planting or in pasteurized soil treatments. A handful of *Glomus* spp. ASVs were, however, identified when plants were grown in live soil (uninoculated control, live + *F.mosseae* and, Live + *C. claroideum*). This suggests that TRECO rootstocks were able to establish new relationships with *Glomus* spp. present in live soil, regardless of the presence of either inoculum. In the rootstocks from Cameron nursery, a variety of *Glomus* spp. existed prior to planting and two of these (ASV 5 and 9)

were maintained at relatively high levels across both live and pasteurized treatments (except in the Live + *F. mosseae* treatment).

Despite representing one of the most promising commercially available products (in terms of the ability to colonize apple seedlings in pasteurized soil; CR 2024), we could find no evidence of successful establishment of *F. mosseae* in pasteurized or live soil, regardless of nursery origin. The *F. mosseae* commercial inoculant did, however, contain a *Claroideoglomus* sp. (ASV 34, Clade 4) which colonized rootstocks in both pasteurized and live soil containing the inoculum. This ASV was identified in both TRECO and Cameron rootstocks and only in the following treatments: Pasteurized + *F. mosseae* and Live + *F. mosseae*. As previously discussed in CR 2022, this represents another example in which what the manufacturer advertises is not always what is actually present.

In addition, ASV 47 (Clade 4) represented a *Claroideoglomus* species that was present in the *C. claroideum* inoculum which successfully formed relationships with G.11 rootstocks from both nurseries in pasteurized and in live soil. This ASV was identified in both TRECO and Cameron rootstocks and only in the following treatments: Pasteurized + *C. claroideum* and Live + *C. claroideum*. Finally, it is worth noting that a handful of "unknown" AMF ASVs closely related to *Paraglomus* species (ASV 84, 86 and 149; Fig 2) were detected in live soil treatments from both TRECO and Cameron nurseries. This finding is in alignment with other sequence-based studies in which *Paraglomus* spp. were detected in apple roots and orchard soil systems of Central Washington (Van Horn, et al., 2021).

Assessing benefits of specific apple rootstock-AMF associations (Obj. 2): **Plant tolerance to water stress.** AMF colonization of G.11 rootstocks was assessed microscopically after 5 weeks. The root systems of plants that had been inoculated with *Rhizophagus irregularis* spores were colonized; 25% and 33% root length colonization in live and pasteurized orchard soil, respectively. At this time, no AMF were detected in uninoculated control plants grown in live or pasteurized orchard soil. During the 4-week water-stress experiment which followed, plant physiological response data, including stomatal conductance and stem water potential, were collected. Stomatal conductance refers to the diffusion of gases (e.g., water vapor) through plant stomata. Dry soil reduces the transpiration of water through a plant. Therefore, stomatal conductance will be higher when plants are *less* water stressed. As expected, G.11 rootstocks cultivated in "live" orchard replant soil with a 30-40% water deficit, had reduced stomatal conductance relative to those cultivated in well-watered soil (~80% field capacity) (Fig. 3). In the presence of replant pathogens (i.e., live orchard soil), *R. irregularis* had a highly significant, positive effect on plant water uptake (i.e., stomatal conductance) under both water-stressed and well-watered conditions ($p = 0.0001$ and $p = 0.0009$, respectively). Therefore, in these treatments (AMF x WW and AMF x WS), water acquisition likely occurred through a combination of direct root uptake and *R. irregularis* hyphal uptake/transfer to plants. This result provides clear evidence of *R. irregularis* directly functioning in a beneficial role in "live" orchard soil and represents a specific AMF-rootstock relationship that could be harnessed to improve drought tolerance.

When cultivated in pasteurized soil, however, colonization by *R. irregularis* had a significant inhibitory effect on stomatal conductance ($p=0.012$) when plants were well-watered and no effect on stomatal conductance when plants were water-stressed. The reason for this result is not clear. To date, microbe-microbe interactions in the apple endosphere/rhizosphere are still poorly understood, and functional outcomes of AMF mycorrhization are likely to depend on complex interactions between environmental conditions and other soilborne microorganisms.

Plant nitrogen acquisition. In the current study, we also assessed whether *R. irregularis* benefited plants in terms of nitrogen uptake. When cultivated in live soil and *well-watered*, plants pre-

colonized by *R. irregularis* recovered significantly more ^{15}N (from the ^{15}N-enriched fertilizer; ammonium-^{15}N nitrate; $^{15}NH_4NO_3$) than non-inoculated plants growing under the same conditions (Fig. 4; p=0.01). Under these conditions (live x AMF x WW), there was almost a doubling in the amount of ^{15}N that the mycorrhizal plants acquired from soil (relative to uninoculated plants; live x No AMF x WW). This amount (6 mg) was similar to the level of ^{15}N acquired when plants were well-watered and grown in the absence of replant pathogens (pasteurized x AMF x WW). When well-watered plants were grown in the absence of replant pathogens (i.e. pasteurized soil), ^{15}N recovery between AMF and non-AMF treatments was not significantly different (Fig. 4). This result indicates that, in live/well-watered soil, colonization by *R. irregularis* can benefit plant absorption of water and nitrogen by helping to compensate for and/or reduce root tissue damage caused by soilborne pathogens.

In *water-stressed* treatments, however, *R. irregularis* did not significantly affect ^{15}N recovery, regardless of presence/absence of soil microbes (Fig. 4). This was surprising considering the significant increase in stomatal conductance when inoculated plants grown in live soil were water-stressed (Fig 3). That said, plant uptake of nitrogen is partly regulated/balanced by the amount of water available in the soil. Therefore, there may have been less of a need to acquire N from soil in water-stressed treatments. Crops can become simultaneously water and nitrogen limited during drought episodes (Plett, et al., 2020); however, in this experiment, water stress did not appear to negatively affect plants' ability to access inorganic nitrogen.

Plant nitrogen partitioning. Compared to well-watered plants, watered-stressed plants generally contained *proportionally* more ^{15}N in roots than in shoots, regardless of soil replant status (Fig. 5). When water limits growth, plants may prioritize/retain nitrogen in roots to maintain vital functions. When experiencing a combination of replant pressure and water-stress, a plant's ability to prioritize root growth over shoot growth may become even more important. In live soil, plants that were pre-colonized by *R. irregularis* partitioned a significantly higher proportion of recovered ^{15}N into roots when water-stressed (AMF x WS) than when well-watered (AMF x WW) (Kruskal-Wallis test, p=0.02; Dunn's multiple comparison tests, p=0.01). In pasteurized soil, a similar trend appeared, but no significant differences were identified between these two treatments. Taken together, results suggest that *R. irregularis* can significantly benefit the ability of G.11 to retain/accumulate nitrogen in root tissue under combined water-stress and replant pressure.

FIGURES:

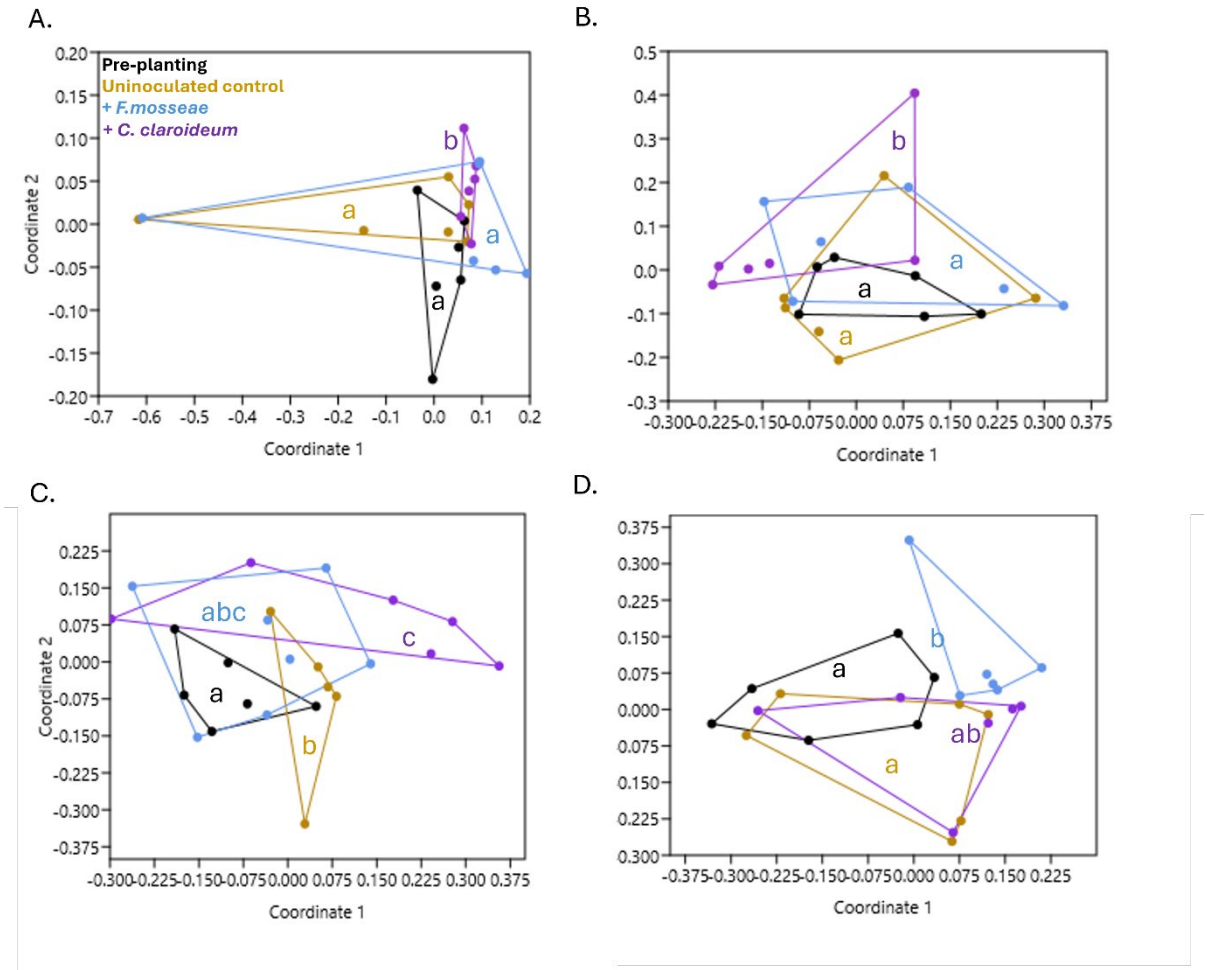

Figure 1. Effect of different treatments on pre-established (nursery-derived) AMF community composition in pasteurized (A and B) vs. live (C and D) orchard soil. Root tissue was collected prior to planting G.11 rootstocks into SRO orchard soil (Pre-planting; Black) and 2 months post-planting into either: uninoculated control soil (Brown), soil inoculated with the AMF *F. mosseae* (Blue), or soil inoculated with the AMF *C. claroideum* (Purple). Convex hulls enclose all samples derived from the same soil treatments. In panels A and C, rootstocks were obtained from TRECO Nursery; in panels B and D, rootstocks came from Cameron Nursery. Ordination of Glomeromycota (AMF) communities was conducted by NMDS analysis of amplicon sequence variant (ASV) data using the Bray-Curtis dissimilarity coefficient. Letter groups indicate significant differences ($p < 0.05$) between treatments as indicated by 1-way ANOSIM conducted using Bray-Curtis dissimilarity coefficient.

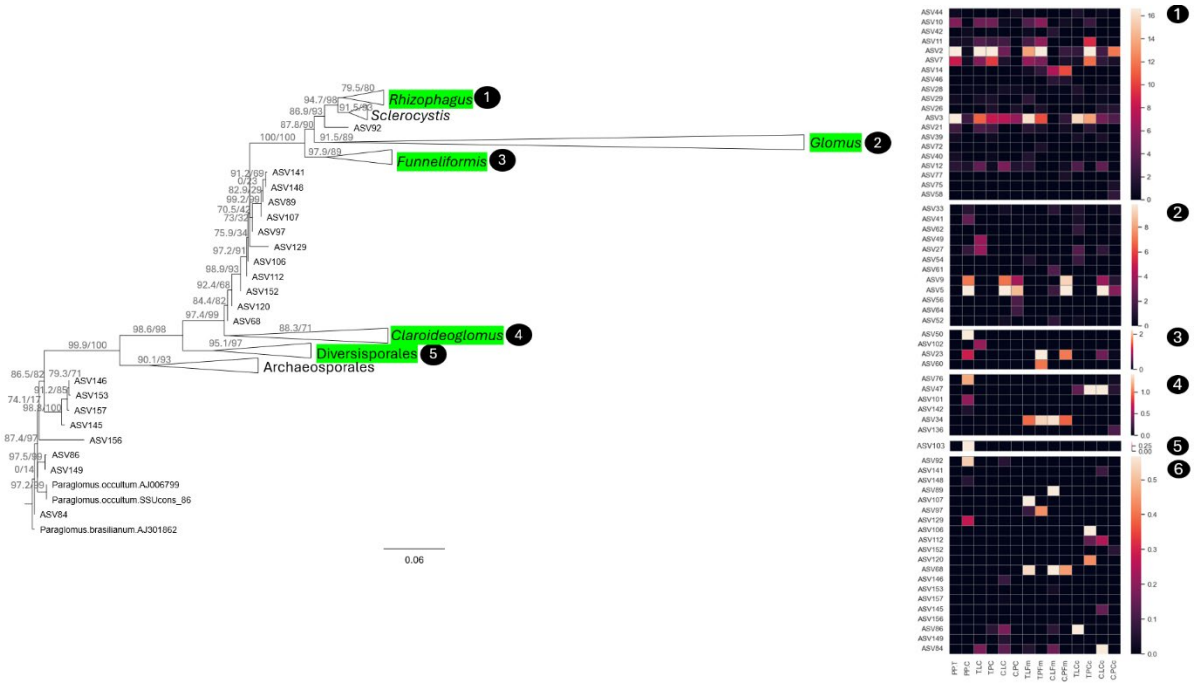

Figure 2. Figure 2. (left) A simplified version of the full phylogenetic tree for Phylum Glomeromycota used to track changes to nursery-derived AMF communities (Obj. 1). Triangles represent clades (i.e., groups of related sequences) that have been "collapsed" in order to visualize tree structure. Triangle length represents sequence divergence within a clade; triangle height represents the number of sequences in the clade. Numbers at each node represent "bootstrap" values (support for each branch). Clades highlighted in green contain amplicon sequence variants (ASVs) recovered from our sequencing analysis along with reference sequences. **(right)** Heatmaps showing relative abundance of ASVs within the major clades shown on the left (labeled 1-5). Group 6 contains ASVs from our sequencing analysis that are not taxonomically annotated (i.e., no reference sequences). Experimental treatments are listed along the x-axis of the heat map: Experimental treatments are listed along the x-axis of the heat map: PP.T and PP.C = TRECO (T) and Cameron (C) pre-planting (PP); TLC and TPC = TRECO live and pasteurized controls; CLC and CPC = Cameron live and pasteurized controls, Fm = +*F. mosseae*, Cc = +*C. claroideum*. The left y-axis lists ASVs identified in the experimental samples; the relative abundance of each ASV is depicted on the right y-axis along a color gradient (the lighter the color, the more abundant the ASV). Values represent the mean of 6 biological replicates for each treatment.

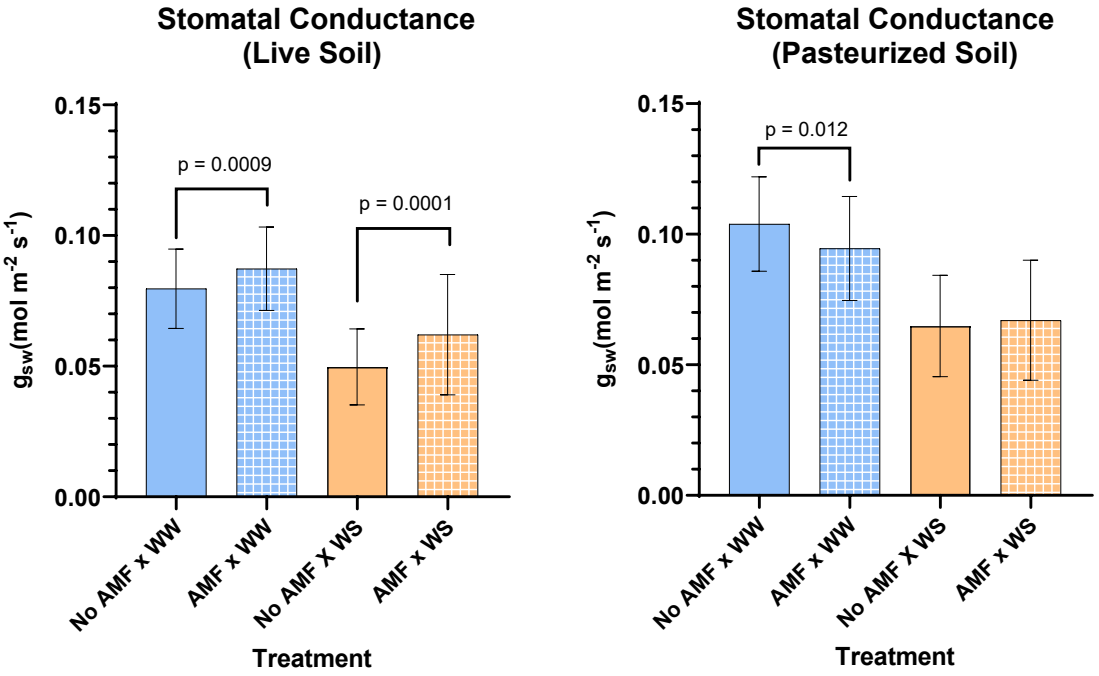

Figure 3: Bar graphs showing mean stomatal conductance by G.11 rootstocks cultivated in live (left) or pasteurized (right) orchard soil with (WS; orange bars;) or without (WW; blue bars) a water deficit (30-40% field capacity). Hatched bars represent treatments in which plants were colonized by the AMF *R. irregularis* prior to experiencing the different watering regimes. For all treatments, stomatal conductance measurements were taken from 6 different plants on the same set of 20 sampling dates (over the course of 1 month). Paired t-tests were used to control for inherent variability between days; treatments experiencing the same watering regime with or without AMF were "paired".

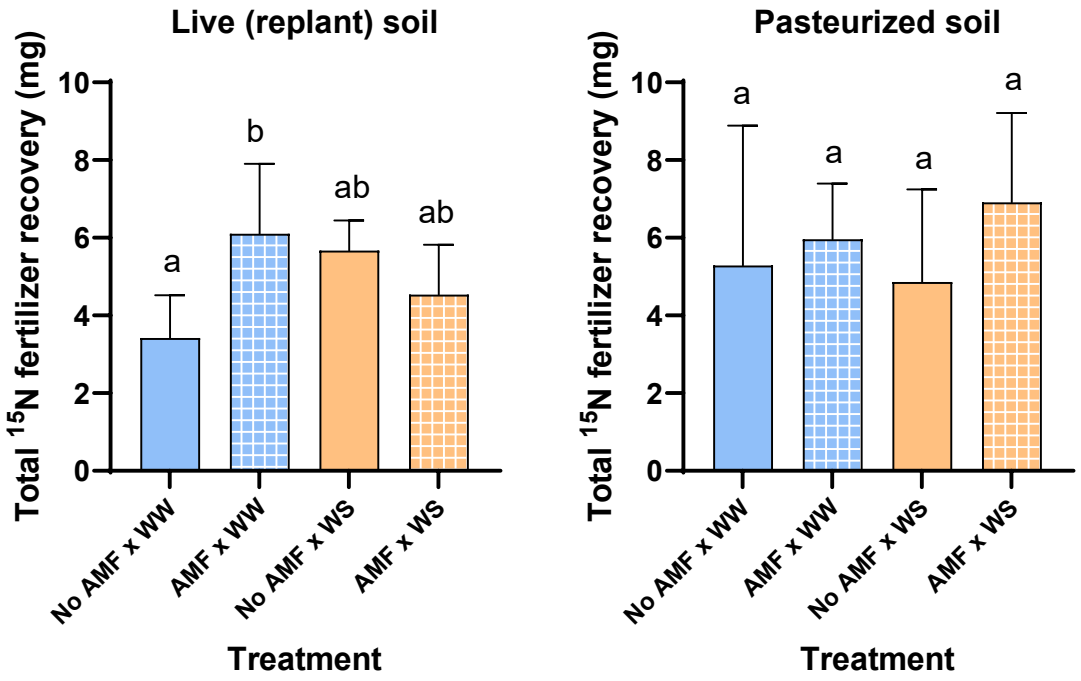

Figure 4. Bar graphs showing total ^{15}N fertilizer recovery into plants cultivated in either live "replant" soil (left) or pasteurized soil (right) after 1 month. Well-watered (WW) treatments are shown in blue, while water-stressed (WS) treatments are colored orange. Hatched bars represent treatments in which plants were colonized by the AMF *R. irregularis* prior to experiencing the different watering regimes; n=6 for all treatment combinations, except Live x AMF x WS where n=5. Within each soil type, statistically significant differences between treatments are represented by different letter groups (Kruskal-Wallis test, Dunn's multiple comparison tests, $p<0.05$).

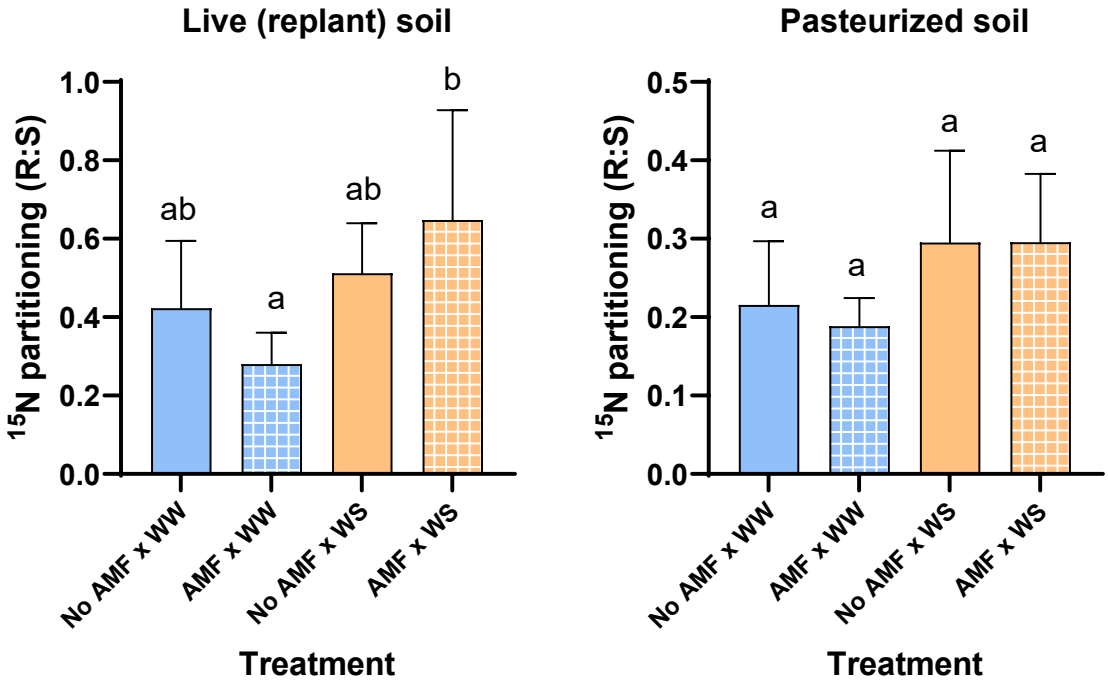

Figure 5. Bar graphs showing partitioning of ^{15}N recovered from ^{15}N-enriched fertilizer into roots vs. shoots (R:S) in G.11 rootstocks cultivated in live (replant) and pasteurized soil under different watering regimes. Well-watered (WW) treatments are shown in blue, while water-stressed (WS) treatments are colored orange. Hatched bars represent treatments in which plants were colonized by the AMF *R. irregularis* prior to experiencing the different watering regimes (n=6 for all treatment combinations, except Live x AMF x WS where n=5). Within each soil type, statistically significant differences between treatments are represented by different letter groups (Kruskal-Wallis test, Dunn's multiple comparison tests, $p<0.05$).

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Executive Summary

Project title: Assessing Barriers to and Benefits of AMF Colonization in Apple

Key words: AMF, apple rootstocks, drought tolerance, nitrogen uptake, replant disease

Abstract: Microorganisms living inside plant tissues (endophytes) often play very specific and crucial roles in promoting the health and growth of their host plant. Arbuscular mycorrhizal fungi (AMF) are root-colonizing endophytes present in most terrestrial ecosystems, including agricultural soils. AMF have been shown to provide a spectrum of benefits to their plant hosts including improved tolerance to water stress, access to nutrients, and disease resistance. Although many AMF species are generalists, functional benefits may be fungus- and plant-species dependent. A primary aim of this research was to assess a range of benefits of specific apple rootstock/AMF associations. Previously identified "compatible" rootstock/AMF combinations leading to rapid establishment of a relationship were selected for testing. Specifically, we tested the ability of two different AMF species (*Claroideoglomus etunicatum* and *Claroideoglomus claroideum*) to enhance plant defense against infection by the fungal replant pathogen *Rhizoctonia solani* in G.41 and G.890 tissue-cultured plantlets. A separate experiment was conducted to test for *R. irregularis-mediated* tolerance to water stress and nitrogen uptake ($^{15}NH_4NO_3$) in G.11 rootstock. Experiments provided clear evidence of AMF species directly functioning in beneficial roles with commercially available apple rootstock genotypes. Colonization of G.11 rootstocks by *R. irregularis* led to significant increases in stomatal conductance in live orchard soil in both water-stressed (30-40% water deficit) and well-watered (~80% field capacity) treatments. This result highlights the role of a specific apple rootstock-AMF associations in maintaining water supply to plants experiencing a combination of water-stress and replant pressure, especially in less vigorous apple rootstocks with relatively small root systems. When grown in *live* orchard soil and *well-watered*, G.11 rootstocks pre-colonized by *R. irregularis* recovered significantly more nitrogen (from ^{15}N-enriched fertilizer) than non-inoculated plants growing under the same conditions. In addition, under combined water-stress and replant pressure (conditions in which a plant's need to prioritize root growth over shoot growth would be expected to be relatively high), inoculation with *R. irregularis* benefited the ability of G.11 rootstocks to retain/accumulate nitrogen in root tissue. Finally, the AMF *C. etunicatum* (but not *C. claroideum*) significantly enhanced plant defense against subsequent infection by *R. solani* in G.41 (but not G.890), relative to uninoculated controls. Results of this study provide insight into specific AMF-rootstock relationships which could be harnessed to improve disease control, drought tolerance and/or sustainability, and represent a first step towards assessing the utility of and improving upon current practices to promote the establishment of mycorrhizal associations in orchard systems.