Fall 2020

Can arbuscular mycorrhizal fungi protect Rubus idaeus from the effects of soil-borne disease and parasitic nematodes?

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Can arbuscular mycorrhizal fungi protect *Rubus idaeus* from the effects of soil-borne disease and parasitic nematodes?

By

Erika Whitney

Accepted in Partial Completion
of the Requirements for the Degree
Master of Science

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Master's Thesis

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Erika J. Whitney

November 20, 2020

Can arbuscular mycorrhizal fungi protect *Rubus idaeus* from the effects of soil-borne disease and parasitic nematodes?

A Thesis
Presented to
The Faculty of
Western Washington University

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Of the Requirements for the Degree
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Abstract

Chemical controls for agricultural pests and diseases can have detrimental effects on human health and the environment. One alternative is to introduce soil microbes, such as arbuscular mycorrhizal fungi (AMF), that can improve crop resilience to pests and pathogens. While many plants form symbioses with AMF, not all crops benefit from inoculation. We conducted three studies that questioned the effect of AMF from various sources on *R. idaeus* growth and resilience to pests/pathogens. First, in a small observational study, we investigated whether AMF colonization of raspberry roots covaried with stand vigor. In two subsequent greenhouse experiments, we asked (1) if AMF inoculation could increase the growth of *Rubus idaeus* cv. Meeker (red raspberry), (2) if AMF inoculation would improve plant resistance to the pathogen *Phytophthora rubi* and parasitic nematode *Pratylenchus penetrans*, and (3) if the source of AMF mattered.

In each greenhouse study, we grew *R. idaeus* with differing AMF inoculum prior to exposing them to pest/pathogens. Plants in the first greenhouse study were inoculated with no AMF, a constructed AMF community, or whole-soil inoculum from the root zone of wild *Rubus parviflorus* or farmed *R. idaeus*. All plants received small microbes (<11 μm) from mixed inocula. After 10 weeks, those plants were challenged with neither, either, or both *P. rubi* and *P. penetrans*. We measured plant biomass and height, shoot nutrients, AMF colonization of roots, and nematode densities. The second greenhouse study was conducted earlier in the spring and with younger plants. Plants first received AMF from the root zone of farmed *R. idaeus,* commercial AMF inoculum, or no AMF. After 5 weeks, half the plants were challenged with *P. rubi*. Plant height, biomass, and survivorship was assessed.

In contrast with our expectations, we found *R. idaeus* farm soil harbored AMF propagules at a similar density and infectivity as wild soil – both colonized 91% of roots despite high soil phosphorous. A lack of biomass or nutrient differences in plants receiving *P. rubi* and *P. penetrans* in the first experiment indicates we did not achieve pest/pathogen densities that impact plant growth. In contrast, conditions more favorable to *P. rubi* in the second experiment led to high rates of pathogen infection. In this experiment, whole-soil inoculum from the commercial farm increased the survival rate of young *R. idaeus* challenged with *P. rubi* by 300%, while commercial inoculum offered no benefit. We found no evidence that mycorrhizal inoculum altered nematode densities in roots or soil. Plants receiving *P. penetrans* had 315-680 nematodes/g root, with 55% lower densities in plants that also received *P. rubi*, suggesting an interaction between these organisms.

We conclude that the soil microbial communities on mature *R. idaeus* farms contain beneficial AMF, and that these biotas increase plant resilience to the pathogen *P. rubi,* at least under greenhouse conditions. These results are a promising step in the development of strategies to promote crop resilience and long-term sustainability of raspberry production.

Acknowledgements

This project was greatly supported by the enthusiasm and support of my advisory committee, and dedicated students who participated in various stages of the project. I owe boundless gratitude to my advisor Rebecca Bunn, PhD, for her unending positivity and support through all stages of the project. Special thanks to Lisa Wasko DeVetter, PhD, for bringing expertise in fruit crops to the project design, and for connecting us with experts and resources ranging from the USDA in Corvallis to the agricultural communities of Whatcom County. Thank you to Jenise Bauman, PhD, for thoughtful contributions to the research design and challenges.

I want to thank Carly Tryon, BS, for her good humor and hard work throughout both years of the project as an undergraduate research technician. Thanks also to Patrick Norman, BS, who conducted the Spring Short Study as an undergraduate intern. I am very grateful to all of the graduate, undergraduate, and high school students who were willingly wrangled into volunteering on various phases of the project. Especial thanks to the long-term volunteers Leila Jones, Hannah Armantrout, Dexter Davis, Maya Newman, and Risa Askerooth. Thank you also to my family and Hans Shepherd, who have always encouraged me to pursue the work I love.

Enormous thanks to the participating farms who allowed us to sample soils and roots from their fields. Thank you to Inga Zasada and Jerry Weiland, for expertise on *Pratylenchus penetrans* and *Phytophthora rubi*, and for the supporting work of your labs. Thank you to the Biology Department for allowing use of your greenhouse and laminar flow hoods. Thank you also to the Institute for Watershed Studies for allowing me to spend almost a hundred hours on your microscope. Without the cooperation of so many people and organizations, this project would not have been possible – it has been a joy working with all of you.

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1. Introduction

Microbiota for Sustainable Agriculture

To feed a growing population with limited arable land will require innovative methods that improve crop productivity in conjunction with long-term soil health. Soil microbes can either help or hinder plant growth, and are considered a largely untapped toolbox for improving plant productivity (Mariotte et al. 2018). Conventional agricultural practices can diminish the biomass, diversity, and species richness of beneficial microbes in the soil—microbes which can support plants via nutrient acquisition, pathogen protection, and more (Banerjee and Anderson 1992, Barrios 2007, Friesen et al. 2011, Köhl et al. 2014, Dangi et al. 2015, Mariotte et al. 2018). Diminished soil microbial biomass and diversity can lead to increased pathogen pressures and increased leaching of nutrients from soil, thus reducing plant productivity over time (Mazzola 2004, de Kroon et al. 2012, Köhl et al. 2014, Mariotte et al. 2018). Managing crops to promote healthy soil communities could vastly improve the sustainability of agricultural ecosystems. Agricultural practices shown to improve crop productivity through effects on soil biota include plant diversification (intercropping or crop rotations), mulching, reduced tillage, and active introduction of key beneficial biota (Mariotte et al. 2018, Bender et al. 2016, Forge and Kempler 2009, Zhu et al. 2000). Each strategy for long-term crop productivity supported by soil microbial communities may not have equal benefits in all cropping systems.

Management of one group of soil biota, arbuscular mycorrhizal fungi (AMF), has high potential to improve agricultural outcomes. Through a symbiosis with plant roots, AMF can promote beneficial soil communities, suppress soil pathogens, and cause changes in plant

nutrition, physiology, and exudation that improve growth and/or reduce disease severity (Fitter and Garbaye 1994, Brussaard et al. 2001, Whipps 2004, Schouteden et al. 2015). Yet, AMF abundance is negatively correlated with land use intensity in agroecosystems around the world (Fester and Sawyers 2011). Given the range of potential benefits of symbioses with AMF, crop inoculation with AMF could be a sustainable strategy to improve resilience to both biotic and abiotic stressors.

Despite great potential to benefit plants, there are concerns that AMF lack a strong role in production agriculture (Ryan and Graham 2002). High nutrient conditions may limit the utility of AMF; soil phosphorous in excess of plant requirements has been shown to inhibit the formation of symbioses between AMF and plants (Kahiluoto et al. 2001, Bittman et al. 2005, Jansa et al. 2009) and to reduce benefits to plant nutrition and biomass (Johnson 2010), yet AMF also influence plant tolerance to abiotic and biotic stressors such as drought and pathogens (Harrier and Watson 2004, Gianinazzi et al. 2010). Furthermore, the quality of symbiosis between plants and AMF is highly dependent upon both species involved, environmental factors, and the soil microflora (Vestberg et al. 1994, Artursson et al 2006, Jacott et al. 2007, Veresoglou et al. 2012), therefore the capacity for AMF to benefit plants under high nutrient conditions could vary as well. Broad generalizations about AMF capacities and limitations may fail to reflect the range of outcomes from their partnership across crops and cropping systems, and focused research is needed to elaborate the potential benefits of AMF within specific cropping systems.

Whole-soil inoculum from local sources may be the most effective means of introducing beneficial AMF for agricultural uses. Utilizing whole-soil inoculum retains the complexity of the

natural system, including bacteria which often act synergistically with AMF to increase plant nutritional or defense responses (Artursson et al. 2006, Adesemoye et al. 2008, Turrini et al. 2018). Studies have further demonstrated that local inocula have more beneficial effects on plant growth than foreign inoculum, due to adaptations to local climate and species-specific interactions (Antunes et al. 2011, Emam et al. 2015, Rúa et al. 2016). Most of these studies demonstrating the benefit of local inoculum have focused on natural systems, so the question remains whether comparable benefits would result from local agricultural soil microbial communities. Disturbances such as fertilization, fumigation, and tillage can reduce AMF taxa richness (Verbruggen et al. 2012) and favor parasitic symbionts (Johnson 2010, Porter and Sachs 2020). In a metanalysis, Hoeksema et al. (2018) brought evidence of coevolution of plants and AMF, which suggests that plants of a similar phylogenetic lineage are likely to respond similarly to a given genera of AMF. Therefore, when considering microbial inoculants for use on cultivated crops, it may be valuable to consider whole soil inoculum from nearby, undisturbed systems that contain phylogenetically similar plant species.

Raspberry - Challenges and Prospects

Red raspberry, *Rubus idaeus,* is a valuable perennial crop in Washington state which faces challenges that likely stem from negatively impacted soil communities. The average productive lifespan of *R. idaeus* plantings in the region has declined from 10-20 years historically to a mere 5-7 years in 2013 (Gigot et al. 2013). Common management practices include tillage, fertilization, and the use of fungicides and pesticides; all of which are known to affect abundance and diversity of beneficial biota (Ibekwe et al. 2001, Mazzola 2004, Dangi et

al. 2015, Hage-Ahmed et al. 2019). While no study has yet assessed whether beneficial microbiota is impacted in *R. idaeus* fields, increases in pathogens and parasitic nematodes have been observed and are likely contributing to the decline in productivity, especially the root pathogen *Phytophthora rubi* and migratory endoparasitic nematode *Pratylenchus penetrans* (Gigot et al. 2013). These organisms damage the fine roots of plants, thus reducing nutrient and water uptake, and can impair the establishment of new plants, stunt plant growth, and reduce crop vigor and yield (Barney and Miles 2007, Gigot et al. 2013, Rudolph and DeVetter 2015, Han et al. 2014).

Of the beneficial biota which may be reduced in this crop system, AMF have a high probability of improving raspberry establishment and resilience to such crop-specific pests and pathogens, whether or not AMF are currently lacking in the field. Early inoculation is advantageous because it can take weeks for plants to establish the symbiotic association with AMF, and inoculation of plants prior to planting in the field has been observed to counter the negative effects of pathogens on growth and yields significantly better than co-inoculation or late-stage inoculation (Forge et al. 2001, Talavera et al. 2001). AMF have been observed to alleviate impacts of both *P. penetrans* and *Phytophthora sp.* in agricultural systems (Talavera et al. 2001, Forge et al. 2001, Pozo et al. 2002, Whipps 2004). However, to our knowledge no studies have yet investigated the interaction of AMF symbioses with *P. rubi* infection, nor whether AMF symbioses improves growth of *R. idaeus* challenged with *P. penetrans.* The effect of mycorrhizal inoculation on establishment and growth of raspberry challenged by either pest or pathogen merits further investigation.

Research Objectives

In a small observational study, we investigated whether AMF colonization of *R. idaeus* roots and stand vigor covaried. Through two subsequent greenhouse experiments, we assessed the following research questions:

1. Can AMF inoculation increase the growth of *R. idaeus*?
2. Can AMF inoculation improve *R. idaeus* resilience to the soilborne pathogen *Phytophthora rubi* or plant-parasitic nematode *Pratylenchus penetrans*?
3. Will plants respond differently to AMF available in managed *R. idaeus* fields compared to other sources?

Due to the tradeoff costs of AMF symbioses (Jacott et al. 2017), we hypothesized that plant growth would be reduced by AMF inoculation under unstressed conditions, but that survival and growth of plants colonized by AMF would be better than uncolonized plants stressed by *P. penetrans* and/or *P. rubi*. Furthermore, we hypothesized that raspberry resilience to pest and diseases would differ based on the source of prior AMF inoculation. Due to evidence that AMF diversity is correlated with improved productivity (Maherali and Klironomos 2007) we hypothesized that plants inoculated with a community of AMF which was built to maximize phylogenetic diversity (8 species representing two orders of AMF) would result in greater biomass production than with either of the other AMF sources. Under the premise that agricultural manipulation and high nutrient conditions impair AMF communities, we hypothesized that AMF of a field soil would lead to lower colonization rates than AMF from a wild soil.

2. Materials and Methods

2.1 Experimental Overview

We began with a Preliminary Observational Study (section 2.2), which investigated whether AMF colonization of raspberry roots and plant vigor covaried. This motivated the "Summer Long Study" (section 2.3) which assessed whether AMF available in farm soil or other sources could affect *R. idaeus* growth or susceptibility to two common problematic soil-borne organisms in Western Washington: the plant-parasitic nematode *Pratylenchus penetrans,* and the pathogen *Phytophthora rubi.* Due to the promising colonization levels observed in the Summer Long Study, but lack of infection by *P. rubi*, we conducted a "Spring Short Study" (section 2.4) to verify whether *P. rubi* would be infective with younger plants under cooler environmental conditions, and to test whether AMF inoculation affected subsequent plant susceptibility.

2.2 Preliminary Observational Study

Within a production field near Lynden, WA, differences in plant vigor were identified and quantified as a difference in the density of fruiting floricanes; averaging only 77 floricanes per 25 ft in the "low vigor" rows, compared with 101 floricanes per 25 ft in the "high vigor" rows (Tim Miller, personal communication).

To assess whether vigor and root colonization covaried, we assessed colonization in roots from these rows. On September 11, 2018, we collected soil from the root zone of plants in 10 rows of each vigor category. Ten samples of soil were dug at regular intervals along each

row, 10 cm wide by 20 cm deep, and combined with the other samples from that row. From combined soils, we then collected fine roots connected to larger woody roots, washed them free of soil, then proceeded to clear, stain, and plate those fine roots following the protocol described in section 2.37. AMF colonization for each root fragment was examined at 200x magnification on a Nikon Eclipse 80i compound microscope. Colonization within each fragment was categorized as 'high' (>50% containing AMF structures), 'low' (<50% containing AMF structures), or 'absent' (no AMF).

2.3 Summer Long Study

The first greenhouse experiment was conducted from May – September 2019. This was a 24-week, full factorial greenhouse experiment (4 AMF x 4 stress, 10 reps; Table 1). During Phase 1 (10 weeks) we compared the growth of unstressed plants inoculated with AMF from three sources to a control, to discern whether raspberries differ in their response to the AMF available in those sources. After that, we exposed those plants to *Pratylenchus penetrans*, *Phytophthora rubi*, neither, or both, to test the impact of the different plant-mycorrhizal associations on disease progression and plant growth under stress during Phase 2 (14 weeks). All plants received small microbes (<11 μm) from mixed inocula.

To account for differences throughout the greenhouse in light, proximity to heating and cooling mechanisms, and other environmental variables, we arranged plants in a randomized block design. One replicate from each treatment was randomly located within each block.

Table 1: Illustrates the number of replicates in each treatment combination. Phase 1 AMF treatments were applied at the start of the experiment, and Phase 2 stress treatments were applied after 10 weeks.

		Phase 2 Stress Treatments			
		No stress	*P. penetrans*	*P. rubi*	*P. penetrans* & *P. rubi*
Phase 1 AMF Treatments	Control Built Farm Wild	16 treatment combinations n=10 each			

2.31 Experimental Parameters

Planting stock

We utilized plugs of tissue cultured *Rubus idaeus* cv. Meeker, provided by the Northwest Plant Co LLC (Ferndale, WA), which averaged 17 cm tall and 0.5 grams dry weight (GDW). 'Meeker' is one of the most commonly grown raspberry cultivars for the Pacific Northwest, accounting for over 70 percent of commercial plant sales (Hoarshi-Erhardt and Moore 2020). As a summer-bearing cultivar with biennial canes, 'Meeker' plants produce vegetative primocanes in the first year, then fruit-bearing floricanes in the second year before they senesce. Once established, this cultivar is somewhat sensitive to root rot (Finn et al. 2014).

Baseline properties of the planting stock were determined by destructively harvesting eight raspberry plugs when the rest were potted into their Phase 1 AMF treatments. On these plants, we separated shoots from roots at the crown, then dried each separately at 60°C for 48 hours in a Heratherm Oven (Thermo Fisher Scientific; Waltham, MA) before weighing on an electronic scale (Denver Instruments SI-4002; Bohemia, NY). A subsample of roots from each

plant were stained and microscopically examined for AMF, following the protocol described in section 2.37. Twelve root segments were assessed per plant.

Figure 1: A plug of *R. idaeus* cv. Meeker from Northwest Plant Co LLC (Ferndale, WA) utilized in the Summer Long Study. Photograph was taken on Day 0, just before plants were potted into their Phase 1 AMF treatments.

Potting Mix

Potting mix used for both phases of the experiment contained a 2:1:1 mixture of farm soil (described in section 2.32), play-sand (Sakrete; Atlanta, GA), and Turface MVP soil conditioner (Turface Athletics; Buffalo Grove, IL). The farm soil was passed through an 8mm diameter USA Standard soil sieve prior to mixing, and the whole blend was steam sterilized twice (80°C, 1 hour) 24 hours apart, using an electric soil sterilizer (SS-30 Electric Soil Sterilizer, Pro-Grow; Phoenix, AZ). Nutrient content of the sterilized potting mix and whole-soil inoculum sources (Table 2) were determined by Exact Scientific Services Inc following protocols described in Miller et al. (2013).

Table 2: Abiotic soil characteristics for final autoclaved potting mix used in each phase of the experiment. Asterisks indicate how nutrient levels compare to normal recommended ranges in agricultural soils. Comparisons for P, K, and pH (Bouska et al. 2018), and OM (Cornell 2020) are raspberry-specific. Comparison for total N is general, sourced from Horneck et al. (2011). No information was available to compare for available N.

	Available N (ppm)	Total N (%)	Bray-P (ppm)	K (ppm)	pH	Organic Matter (%)
Potting mix (Phase 1)	15.1	0.07*	351**	191**	6.78**	1.65*
Potting mix (Phase 2)	16.8	0.08*	280**	227**	6.50**	1.18*
Farm inoculum	12.7	0.15	579**	253**	7.40**	3.20
Wild inoculum	8.29	0.33**	237**	345**	6.04**	9.19**

* *Lower than normal range*
** *Higher than normal range*

Greenhouse Conditions

In both phases of the Summer Long Study, plants were grown in a greenhouse set to maintain temperatures between 15.6 – 21.1°C (60 – 70°F), though temperatures fluctuated from 15 – 43.3°C (59 – 110°F) and averaged 21.7°C (71°F). Average relative humidity was 62%. Light averaged 262 ± 24 μmol/m^2/sec at midday. Plants received at least 12 hours of light per day: supplemental lighting was on a 12:12 period cycle while natural light was longer than that, increasing toward the summer months. Detailed environmental data are in appendix A.

Nutrients

Using the strategy of Taylor and Harrier (2000), we gave plants only water for the first 6 weeks to promote AMF colonization. We then fertilized weekly with 10 – 30 mL of Hoagland's nutrient solution modified to be phosphate-free. At 1X concentration, the modified Hoagland's included:

5 mM $Ca(NO_3)_2$, 5 mM $K(NO_3)$, 2 mM $MgSO_4$, 1 mM Fe(III)EDTA, 46.3 μM H_3BO_4, 9.1 μM $MnCl_2 \cdot 4H_20$, 8 μM $ZnCl_2$, 4 μM $CuCl_2 \cdot 2H_20$, 1 μM $Na_2MoO_4 \cdot 2H_20$

The volume and concentration of nutrient solution was increased in response to signs of nutrient stress. Each plant received 153.3 mg N total over the course of the experiment.

Water

Plants were drip irrigated using ½ GPH emitters (31.6 mL/minute). Water volume and frequency was increased over the course of the experiment to maintain moist but not saturated soils (except during Phase 2, detailed in section 2.33).

2.32 Phase I

The raspberry plugs were potted into 650 mL Deepots (D40H; Stuewe and Sons Inc., Tangent, OR) using one of four AMF treatments described below, and the sterile potting mix described above. The AMF treatment was sandwiched between sterile soil, where plant roots would quickly encounter the inoculum (Figure 2). To homogenize the microbial community smaller than 11 µm across treatments, all plants received 10 mL of a "microbial wash" prepared by blending equal volumes of all AMF inocula with distilled water and filtering the slurry progressively down to 11 µm. The final filtrate was passed through 11 µm filter paper three times to ensure exclusion of AMF (spores and colonized root fragments).

Figure 2: Diagram of potting method for Phase 1 AMF treatments. One of four AMF treatments (white band) was located directly below each plant plug (black cylinder), sandwiched between sterile potting mix (grey fill). Small microbes were applied to all plants as an aqueous mixture, so the microbial community <11 µm would be consistent between treatments.

Description of AMF Treatments

1. Control: No AMF inoculum

2. Built: 10 g per plant of an AMF blend which is a phylogenetically diverse community of AMF species. High species diversity may correlate with functional diversity, and thus increase potential benefits to plants (Verbruggen and Kiers 2010). This blend contained spores of 8 species from 2 orders in sand as a carrier.

Each species in the blend was obtained by the MPG Ranch (Florence, MT, USA) as a pure culture sourced from the International Culture Collection of Vesicular Arbuscular Mycorrhizal Fungi (INVAM). The species were chosen to represent a diverse phylogeny, while selecting fairly cosmopolitan species that had morphologically distinct spores to facilitate visual analyses of community composition. Each pure culture was increased by pot culture on *Plantago lanceolata* from May to August 2018 at the MPG ranch in Florence, MT, USA (46.673 N, 114.016 W). A combined blend of these spores in sand was sent to our lab, and in March 2019 we extracted and quantified spores by species (methods below) to obtain initial relative abundance of each species (Table 3).

Table 3: Abundance of spores within the Built inoculum, by species. Data are means ± standard errors from five sample extractions.

AMF Species	Spores / 10g Inoculum
Claroideoglomus etunicatum	80 ± 26
Claroideoglomus lamellosum	307 ± 49
Dentiscutata heterogama	850 ± 81
Funneliformis mosseae	303 ± 69
Gigaspora albida	13 ± 3
Gigaspora rosea	3 ± 3
Rhizophagus irregularis	460 ± 162
Rhizophagus sinuosum	7 ± 3

3. Farm: 30 g per plant of whole-soil inoculum containing colonized root fragments, fungal hyphae, and spores from the root zone of commercially cultivated *R. idaeus*, which would represent agriculturally managed soil.

We selected a commercial farm in Whatcom County, WA, USA (48.938 N, 122.537 W; elevation 24m) which undergoes management typical for commercial raspberry growers, specifically the use of fumigation to control pests, application of conventional fungicides and insecticides, and fertilization to support plant growth. Importantly, this farm had no history of root-rot nor *P. penetrans* infection. At the time of soil collection, the commercial planting consisted of 18-month old 'WakeHaven' raspberries in raised beds. In June 2017, approximately 21 months prior to our soil harvest, the soil had been bed fumigated with Telone C-35 (Dow Agrosciences, Indianapolis, IN), and broadcast fertilized after fumigation (11N–52P–0K; 145 kg·ha−1). The soil at this site is mapped as a Laxon loam: a coarse-loamy, moderately well drained soil formed from volcanic ash, loess, and glacial outwash (Soil Survey Staff, 2020).

4. Wild: 30 g per plant of whole-soil inoculum containing colonized root fragments, fungal hyphae, and spores from the root zone of a mature stand of Thimbleberry, *Rubus parviflorus,* which would represent an undisturbed soil of the closest wild relative of *R. idaeus* occurring in our region.

We selected a 20-year-old patch of *R. parviflorus* naturally occurring in a clearing adjacent to the forested Sehome Hill Arboretum and Western Washington University (Whatcom County, WA; 48.728 N, 122.486 W; elevation 74 m). The patch measured 3 m in diameter and more than 1.5 m tall. Soils at this site are mapped as a Squalicum-urban land complex, which is Squalicum soil among urban developed areas. Squalicum soil is a gravelly loam, moderately well-drained and formed from volcanic ash, loess, and alluvium over glacial till (Soil Survey Staff, 2020).

Figure 3: Photos of the sources where each whole soil inoculum was collected. The Farm treatment came from a commercial planting of *Rubus idaeus* cv. WakeHaven near Lynden, WA (A). The Wild soil came from an uncultivated, 20-year old patch of *Rubus parviflorus* in Bellingham, WA (B).

Preparation of whole soil inoculum

We collected the Farm and Wild whole-soil inoculum on March 14th and March 15th 2019, respectively. At each site, we used a square blade shovel to remove 10 cores (25 x 25 x 25 cm) from the root zone of several plants within a single thimbleberry patch (Wild) or within a single row of raspberries (Farm), respectively. Replicate cores were brought immediately back to the lab and sieved using an 8 mm soil sieve to remove large chunks of debris, then mixed thoroughly. Soil was stored at 4°C until use.

Determination of AMF spore density

To assess initial differences in inoculum density, AMF spores were extracted from 10 g soil samples (fresh weight) following a sucrose gradient method adapted from Allen et al. (1979) and Ianson and Allen (1986). We suspended fine sediments and spores by repeat agitation of the soil in a generous amount of water, and concentrated the suspended fine fraction by filtering through a 250 µm USA Standard Testing Sieve onto a 25 µm sieve. Spores retained in the large fraction on the 250 µm sieve were collected in a petri dish and counted. The spores and soils were rinsed from the 25 µm sieve into a 50 mL centrifuge tube and pelletized by centrifugation and then the spores resuspended and separated from soils by centrifugation in 2M sucrose solution (Allen et al. 1979). Rather than using a separatory funnel, we then extracted the spores from aqueous solution by vacuum filtration onto GVS Magna^{TM} Nylon Membrane Filters: 10 µm pore size (Bologna, Italy), prepared with 5 mm^2 gridlines. During vacuum filtration, the walls of the funnel were rinsed with deionized water to reduce electrostatic adhesion of spores.

Spores were immediately quantified using a Leica S6 E stereomicroscope (Leica Microsystems; Wetzlar, Germany). For plants inoculated with Built AMF, we could identify the unique spores to species. For all other treatments, AMF spores were categorized by size and color. Size categories utilized were small (<100 microns), medium (100-150 microns) and large (>150 microns).

We found that the Built inoculum contained approximately 6X the AMF spore density of the whole-soil communities (Table 4), though we could not quantify other sources of AMF inoculum such as live hyphae and colonized root fragments which would be present in whole-soil but not in the Built inoculum. To make the initial inoculum density more consistent between treatments, we decided to apply 3X more whole-soil inoculum than Built inoculum, by volume.

Table 4: Spore density of AMF in each inoculum source used in the Summer Long Study. Spore density measures are means ± standard error from 5 replicate samples. Spores per pot were estimated based on the application rate; 30 g/pot for Farm and Wild, 10 g/pot for Built. Farm and Wild also contained AMF propagules in the form of hyphae and colonized root fragments (not quantified).

Inoculum	Spores/g dry soil	Spores/pot
Built	202 ± 10 a	2023 ± 103
Farm	2 ± 0.2 b	50 ± 5
Wild	5 ± 0.4 b	150 ± 13

2.33 Phase 2

After 10 weeks, ten plants from each Phase 1 AMF treatment were introduced to one of four Phase 2 stress treatments with which they would grow for another 14 weeks. At the start of Phase 2, plants averaged 30 ± 4 cm tall.

Phase 2 Stress Treatments

1. Control: potted into sterile soil
2. Phytophthora: potted into soil containing the pathogenic oomycete *P. rubi*
3. Nematode: potted into sterile soil, then received root-lesion nematodes, *P. penetrans*
4. Both: potted into soil containing *P. rubi*, then received *P. penetrans*

Phytophthora treatments: Jars of vermiculite, V8 broth, and oat mixture were prepared as described in Stewart et al. (2014), and autoclaved for 55 minutes at 120°C. After cooling for 24 hours, half of the jars were each inoculated with five 6-mm segments of *P. rubi* on PARP media plates (F-145; USDA, Corvallis), while the other jars were kept sterile. All jars were incubated in the dark at room temperature for 4 weeks, shaken once a week to redistribute the hyphae, as recommended by Stuart et al. (2014). The jars of cultivated *P. rubi* were mixed with sterile potting mix resulting in an approximate inoculum to soil ratio of 1:10 (vol/vol). Similarly, the jars containing only growing media were mixed with sterile potting mix resulting in a 1:10 ratio (vol/vol).

We carefully removed plants from their Deepots so the soil plug could be dropped whole into 1000 mL Mini-Treepots (MT310; Stuewe and Sons Inc., Tangent, OR) filled with

either the sterile potting mix or the *P. rubi* potting mix. Following transplanting, plants were overhead watered to saturation, then returned to drip irrigation that would saturate plants four times daily. At two-week intervals, plants were flooded to encourage infection from *P. rubi* (July 16th – 18th, and July 29th – August 2nd).

Nematode treatments: Nematodes were extracted from the roots of established raspberry plants, sourced from several Whatcom county raspberry farms. We extracted nematodes via aerated incubation in 1000 mL beakers, using roots rinsed free of adhering soil to limit the presence of other nematode species. For each extraction, a handful of roots were rinsed thoroughly, then chopped into 1 cm segments and wrapped in a mesh basket. These bundles were then submerged in deionized water for 3-4 days in low light, air was introduced via bubbling with a Whisper40 Air Pump (method adapted from Barker (1985) and EPPO (2013)). Nematode densities from each round of extractions were calculated by taking five replicate 2 mL samples and counting the number of moving nematodes using a gridded McMaster slide at 100x magnification on a Nikon Eclipse 80i compound microscope.

We applied nematodes twice weekly until each pot in the nematode treatments received 1000 nematodes total. Each application consisted of a tap water suspension of nematodes pipetted into two holes at the base of each plant, and occurred between June 13th to July 18th, 2019.

Midpoint Harvest

To obtain midpoint biomass and root colonization data, we destructively harvested three plants from each AMF treatment on the same day as the rest were introduced to Phase 2 stress treatments. Shoots and roots were dried separately for 48 hours at 60°C, and weighed on an electronic scale. A subsample of fine roots from each plant were cleared, stained, and inspected for AMF colonization as described in section 2.37.

2.34 Growth Measures

We measured above-ground features of each plant every 2 weeks: including plant height, leaf chlorophyll, and the number of nodes.

- Plant heights were measured in centimeters from the base of the plant to the furthest distal end of the leaf tip.
- Leaf chlorophyll was measured with a SPAD-50 Chlorophyll Meter (Spectrum Tech Inc.; Aurora, IL), recording an average of 5 locations from the third fully expanded leaf.
- Nodes were counted whether or not active leaves were present.

2.35 Destructive Harvest

Harvest occurred from September 16th to September 27th. One block at a time, we removed plants from their pots, and gently shook plants to collect soil from roots. We then carefully rinsed roots in cold water to remove any remaining soil. Tops of plants were separated from the roots at the crown, and samples were removed and weighed for various analyses as described below.

2.351 Shoots

Shoot Biomass: Shoots were weighed, placed in paper bags and dried for at least 48 hours at 60°C, then weighed on an electronic scale.

Plant Tissue Nutrients: After drying, shoots (leaves and stems) were ground and then shipped to Brookside Laboratories, Inc. (New Bremen, OH) for tissue analysis. Analyses were performed following standardized protocols outlined in Miller et al. (2013) – total carbon and nitrogen were determined using a C/N combustion analyzer (method P2.02), while all other elements were assessed following the nitric acid and hydrogen peroxide digestion method (method P4.30).

2.352 Roots

Fine Root Sampling: Samples of fine roots were cut from five separate locations around the root mass, combined and the wet weight determined. A cross section of this subsample was frozen at -10°C for later molecular analysis (data not presented). The remainder of the subsample was divided into two replicates to be assessed for mycorrhizal colonization.

Nematode Sampling: For those plants which received nematodes, approximately 8 g of roots were removed by cutting with sterile scissors straight up from the bottom to the top of the root mass, so that roots at every depth would be represented in the sample. This sample of roots was weighed and placed in a Ziploc bag with 100 cm^3 of soil from that plant's pot. These combined samples of root and soil were refrigerated at 4°C for less than a week before mailing

to the Zasada Laboratory in Corvallis for analysis. Following the methods described in Zasada et al. (2015), root densities were measured by extracting nematodes from fine roots by misting. Soil densities were assessed using the Baermann funnel method.

***Phytophthora* Sampling:** To assess infection by *P. rubi*, we sent root and shoot samples of 24 plants to the USDA in Corvallis for re-isolation of *P. rubi*. Two replicates from each treatment combination containing *P. rubi* (Blocks 7 & 8), and one replicate from each treatment without *P. rubi* (Block 7) were sent. Following all other subsampling, the upper 6 cm of roots were separated from the rest and weighed, then placed in a Ziploc bag. The stalk was cut 15 cm from the base, and the lower portion was weighed and included in the same Ziploc bag. Samples were stored at 4°C for less than 4 days before shipping. At the USDA Corvallis, surface sterilized root & shoot fragments were plated onto selective media (PARP) and examined for *P. rubi* growth.

Root Biomass: After all subsamples had been removed and weighed, the remainder of the roots were weighed, and placed in a paper bag. These were dried at 60°C for 48 hours and re-weighed.

2.36 Reconstructing Biomass

Dry weights of samples which were removed for other analyses were estimated by constructing a linear regression of Dry (g) ~ Wet (g) from closely related plant samples. Presented biomasses

are the addition of weighed dry biomass plus estimated dry biomass of any subsamples removed prior to drying.

$Biomass = DW_{measured} + DW_{estimated}$

Linear Models

The best fit linear models used to estimate the weight of each subsample are as follows:

Nematode subsamples: To estimate the dry biomass of the roots that were sent away for nematode analysis, we used a regression on the wet weights of all root samples, excluding blocks 7 - 9 which were not representative because they had disproportionately fewer woody root segments after their upper root mass was sent away.

Dry Weight [nematode subsample] = 1.179 + 0.164(Wet Weight)

($R^2 = 0.78$)

Fine root subsamples: To estimate the dry biomass of the fine roots that were partitioned for various samples, we used a regression on the roots from blocks 7 – 8 which had their upper root mass sent away, including the bulk of their woody roots.

Dry Weight [fine roots subsample] = 0.434 + 0.207(Wet Weight)

($R^2 = 0.93$)

Phytophthora subsamples: To estimate the dry weight of root chunks and stem segments sent for *P. rubi* re-isolation, we replicated the sampling on 24 additional plants (the remainder of Block 7 and all of Block 9) and dried those to build the regression.

Dry Weight [upper roots subsample] = 0.539 + 0.159(Wet Weight)

(R^2 = 0.79)

Dry Weight [lower stem subsample] = 0.199 + 0.429(Wet Weight)

(R^2 = 0.69)

2.37 Mycorrhizal Colonization

To clear pigment from roots, samples were soaked in 3% potassium hydroxide (KOH) at 40°C for 2 – 5 hours. Samples were then rinsed 3X with deionized water, followed by soaking in 5% hydrochloric acid (HCl) in water overnight at 4°C. Roots were then stained for 12 hours with 0.05% w/v trypan blue in lactoglycerol (1:1:1 lactic acid, glycerol and water). This clearing and staining process was adapted from Brundrett et al. (1996). Samples were destained for a minimum of 24 hours in lactoglycerol at 4°C, then were stored in fresh lactoglycerol at 4°C until slides could be prepared. One slide was prepared for each plant; twelve 1-cm root segments were mounted on each slide. We counted colonization using the magnified intersections method (McGonigle et al 1990). Slides were viewed at 200x on a Nikon Eclipse 80i compound microscope and approximately 72 intersections were assessed for each plant.

2.38 Spore production

Soil of each plant was homogenized within their unique treatment combinations, and 500 cm^3 from each treatment was dried in paper bags at room temperature. We followed the same spore extraction method as before, except that only 1 g dry soil was utilized in each extraction. For plants inoculated with Built AMF, we knew the relative abundance of each AMF species in the initial inoculum and could identify the spores to species. For all other treatments, AMF spores were categorized by size and color.

2.4 Spring Short Study

The second experiment was a 15-week, full factorial greenhouse experiment (3 inoculum x 2 pathogen, 10 reps; Table 5) conducted from February to May 2020. We inoculated plants with Farm soil, commercial AMF inoculum, or no AMF, then after 5 weeks we exposed half of those plants to *P. rubi*, to test the impact of the different plant-AMF associations on disease progression and plant growth. We arranged plants in a randomized block design with two replicates from each treatment randomly located within each block.

Table 5: In the Spring Short Study 10 plants received each combination of AMF treatment and stress treatment. AMF treatments were applied at Week 0. Stress treatments were introduced at Week 5.

		Stress Treatments	
		Control	*P. rubi*
AMF Treatment	Control Mykos Farm	6 treatment combinations n=10 each	

2.41 Experimental Parameters

Planting stock

We utilized 2 cm tall tissue culture *Rubus idaeus* cv. Meeker plantlets in agar provided by the Northwest Plant Co LLC (Ferndale, WA) (Figure 4).

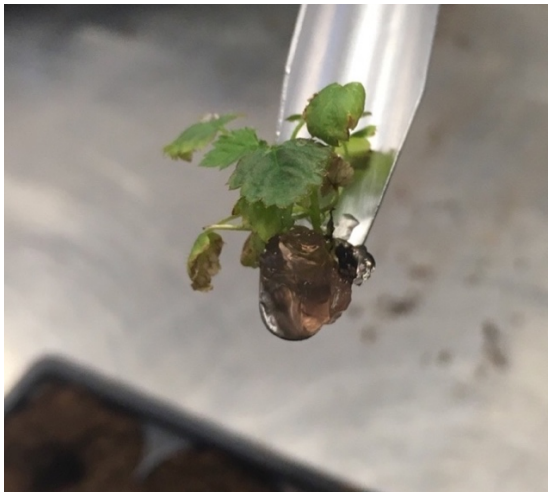


Figure 4: Photo of a tissue culture plantlet utilized in the Spring Short Study. Each plantlet was planted with agar retained around developing roots.

Potting Mix

Potting mix was prepared as described for the Long Summer Study.

Greenhouse Conditions

The average daily range was 17.8 – 25°C (64 – 77°F), with an overall average of 20°C (68°F) over the experiment. Average relative humidity was 48%. Before pathogen introduction, light averaged 161 ± 87 μmol/m^2/sec at midday. Plants received at least 12 hours of light per day: supplemental lighting was on a 12:12 period cycle, with natural light increasing toward the summer months.

Nutrients

We gave plants only water for the first 5 weeks to promote mycorrhizal fungal colonization. After that, we fertilized weekly with 30 mL of Hoagland's nutrient solution, again modified to be phosphate-free. Each plant received 63 mg N total over the course of the experiment.

Water

Plants were watered for 1 minute every 12 hours using a drip irrigation system with ½ GPH emitters (31.6 mL/minute).

2.42 AMF Treatment (5 weeks)

This experiment started on February 6, 2020; when we planted tissue culture plantlets into 6 x 2 seedling trays (150 mL wells). Each plantlet was carefully lifted with a square of agar remaining around its base (Figure 5) and planted into a well containing sterile potting mix with one of the three AMF treatments described below. The AMF inoculum was again layered beneath the plants and between sterile soil as depicted in Figure 2. This time, no microbial wash was applied to the plants. AMF treatments were as follows:

1. Control: No AMF inoculum

2. Mykos: 1 cm^3 per plant of Mykos™ commercial mycorrhizal fungal additive, which contained 300 propagules/gram *Rhizophagus intraradices*

3. Farm: 10 cm^3 per plant of whole-soil inoculum containing colonized root fragments, fungal hyphae, and spores. Soil was sampled from the same row of *R. idaeus* utilized in the Summer Long Study, which contained approximately 2 ± 0.2 spores per gram soil.

2.43 Stress Treatment (8.5 weeks)

After growing with AMF treatments for 5 weeks, plants were then potted into 650 mL Deepots (D40H; Stuewe and Sons Inc., Tangent, OR) containing either sterile soil mix or *P. rubi* infested soil mix (prepared as in section 2.33). At this stage, plants averaged 4.9 cm tall (0.06 GDW).

Figure 5: This photo was taken at Week 5 of the Spring Short Study, immediately after these *R. idaeus* were potted into their stress treatments.

2.44 Response Measures

Starting at Week 5, we measured plant height and assessed survivorship every 1-2 weeks. When plants had fully wilted and begun to dry, they were deemed dead and the date of death recorded (Figure 6). After 8.5 weeks had passed (May 12, 2020) we harvested the surviving plants to assess dried biomass. We separated roots from shoots on each plant and dried these samples at 60°C for one week in a Heratherm Oven before weighing on an electronic scale.

Figure 6: This photo illustrates a plant considered 'dead' following inoculation with *P. rubi* in the Spring Short Study; it is clearly wilted and the leaves mostly dried.

2.5 Statistical Analyses

Sensitivity analysis was performed in G*Power Statistical software (Faul et al. 2007) and all other analyses were performed in R 3.6.0 (R Core Team 2020) via the RStudio user interface (R Studio Team 2020).

Preliminary Observational Study

Due to highly unequal variances and abnormally distributed data, we used the Kruskall Wallace nonparametric test to assess whether there was a statistically significant difference in the percent of colonized root fragments from each plant vigor category.

Sensitivity Analysis

We ran a sensitivity analysis on the Summer Long Study design to define the effect size (Cohen's f statistic) that could be detected for each response variable, given our sample size (n = 160), alpha of 0.05, and desired power of 0.8. We then compared the sensitivity of the

research design with the actual effect sizes observed—presented as Cohen's partial f statistic, which takes into account the distribution of variance among multiple independent variables.

Theoretical effect sizes our design could have detected were found using G*Power Statistical software (Faul et al. 2007). The study was parameterized as a 2-way ANOVA; AMF treatment was a factor with 4 levels (Control, Built, Farm, Wild), and stress treatment was a factor with four levels (Nematode, Phytophthora, Both, and Neither). Observed effect sizes were calculated using the "effectsize" package in R (Ben-Shachar et al. 2020).

Mixed Models

All mixed models were constructed in the R package "lme4" (Bates et al. 2015). Mixed models included Block as a random effect and all explanatory variables of the experimental design as fixed effects.

First, to determine whether the explanatory variables helped to predict the response variable (null vs full model), and whether there was an interaction between stress and AMF treatments worth retaining in the model (full vs interaction models), I used Chi-Square goodness of fit tests and residual plots. For example, the null, full, and interaction models tested for the Summer Long Study were:

- **Null Model:** Response ~ $\mu + (1|\text{Block})$
- **Full Model:** Response ~ $\mu + MTmt + PhyTmt + NemTmt + PhyTmt{:}NemTmt + (1|\text{Block})$
- **Interaction Model:** Response ~ $\mu + MTmt + PhyTmt + MTmt{:}PhyTmt + NemTmt + MTmt{:}NemTmt + PhyTmt{:}NemTmt + MTmt{:}PhyTmt{:}NemTmt + (1|\text{Block})$

With the optimal base model, we then varied the error terms to assess whether random slopes or intercepts improved the model fit, again comparing models using a Chi-Square goodness of fit test. Once the best model was fit, we assessed the residuals to confirm that they were normal and randomly distributed before proceeding to use a Type II Wald Chi-Square goodness of fit test (Venables and Ripley 2002) to determine which variables were significant predictors of the response.

Contrasts

Using the best fitted mixed model, we then ran post-hoc comparisons to find which treatment combinations of interest differed. Type I error was limited by using the Tukey single-step method within the "multcomp" package (Hothorn et al. 2008). When there was a significant interaction between stress or AMF treatments, simple main effects contrasts were used to determine which of those combinations had a significant response. These contrasts were done using the "emmeans" package in R (Lenth 2020).

3. Results

3.1 Preliminary Observational Study

Preliminary root assessments revealed that within a single Lynden farm, higher plant vigor was associated 32% with higher rates of mycorrhizal colonization (Kruskall-Wallace, $\chi 2 = 4.6$, $p = 0.03$). While 92% of root fragments from the "high vigor" rows (n=42) contained AMF colonization, merely 60% of fragments from the "low vigor" rows (n=37) were colonized.

3.2 Summer Long Study

AMF Colonization Potential

Abundance and structural characteristics of root colonization differed between the AMF treatments. Despite differences in initial propagule density (Table 4), both whole-soil inoculum sources resulted in 91% of fine roots containing AMF (hyphae, arbuscules, and/or vesicles), while plants inoculated with AMF from the Built community had 15% lower colonization (Tukey HSD, $p < 0.01$; Table 6). Roots colonized by Built AMF had the highest density of arbuscules (Tukey HSD, $p < 0.001$). In contrast, the highest abundance of vesicles occurred in plants with AMF from Farm and Wild soils (Table 6). Stress treatments did not significantly affect the total colonization levels observed within any AMF treatment (Wald Chi-Square, $\chi 2 < 2.82$, $p > 0.093$).

Table 6: AMF colonization observed in fine roots of *R. idaeus* cv. Meeker grown with different AMF inocula for 24 weeks. Data displayed are mean percent of intersections ± standard errors from 28 replicate plants. Approximately 72 intersections were assessed per plant. Letters indicate significantly different means (Tukey HSD, $p < 0.05$).

AMF Treatment	% Hyphae only	% Vesicles & Hyphae	% Arbuscules & Hyphae	% Vesicles, Arbuscules, and Hyphae	Total % AM
Control	40 ± 4 c	6 ± 2 b	9 ± 2 b	0 ± 0 b	55 ± 6 c
Built	55 ± 2 b	3 ± 1 b	17 ± 2 a	0 ± 0 b	76 ± 2 b
Farm	55 ± 2 b	22 ± 2 a	11 ± 1 b	3 ± 1 a	91 ± 2 a
Wild	66 ± 3 a	15 ± 2 a	8 ± 2 b	2 ± 1 ab	91 ± 1 a

Though control plants were colonized by the end of the experiment, a lack of relationship between stress treatments and colonization levels detected in those control plants (Wald Chi-Square, $\chi 2 < 0.92$, $p > 0.34$) indicates that the AMF contamination was consistent across all treatments. AMF spores extracted from soils of control plants were dominantly small-medium sized (Figure 7).

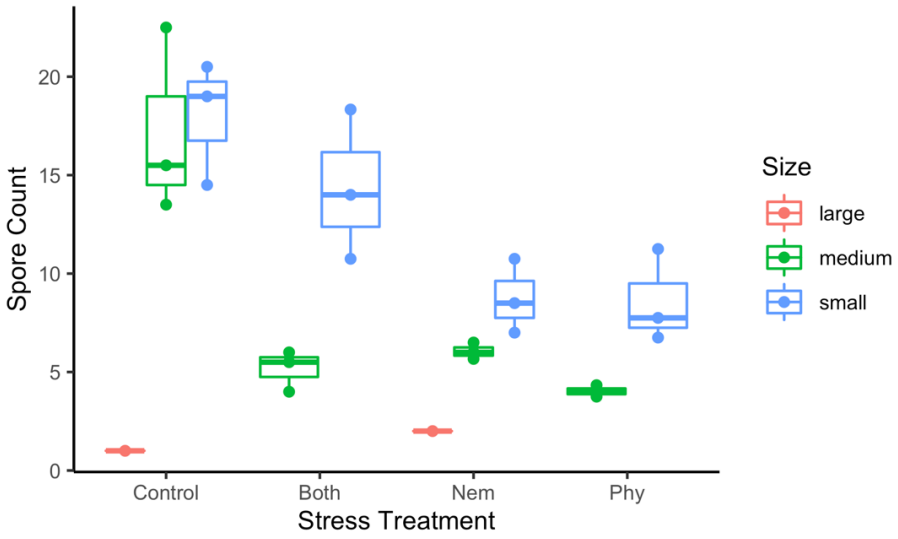


Figure 7: Spore counts per gram of dry soil, extracted from the AMF Control treatment, grouped by Phase 2 stress treatments. Points indicate counts from each of 3 samples taken from pooled soils; boxplots represent median and interquartile range of those three samples. Spore sizes were categorized as small (<100 microns), medium (100-150 microns) and large (>150 microns).

Plant Growth

Overall, *R. idaeus* inoculated with AMF tended to be as tall or shorter than control plants, had equivalent or less total biomass, and had higher leaf chlorophyll than uninoculated controls. Plants with Wild AMF were the most reduced in size, averaging 18 ± 3 cm shorter than control plants (Table 7). The number of nodes on *R. idaeus* canes was related to cane length, but the density of nodes did not differ between AMF Treatments (Appendix H). Plants with Farm and Wild AMF had on average 8.5% less root biomass than plants with Built AMF and Control plants (Table 7). Aside from the difference in root biomass, plants with AMF from the Built community were similar to those with Farm AMF (Tukey HSD, $p>0.05$; Table 7).

Table 7: Physical features of 24-week old harvested 'Meeker' raspberry plants. Data displayed are means ± standard errors from 40 replicates, averaged over Phase 2 stress treatments. Different letters indicate significantly different means (Tukey HSD, $p < 0.05$).

AMF Treatment	Shoot mass (g/plant)	Root mass (g/plant)	Total Biomass (g/plant)	Chlorophyll (SPAD)	Height (cm)
Control	12.6 ± 0.2 a	6.4 ± 0.1 a	19 ± 0.3 a	31 ± 0.2 b	145 ± 4 a
Built AMF	12 ± 0.2 ab	6.2 ± 0.1 a	18.2 ± 0.2 ab	33 ± 0.2 a	141 ± 3 a
Farm AMF	11.8 ± 0.3 b	5.8 ± 0.1 b	17.6 ± 0.3 bc	33 ± 0.3 a	138 ± 4 ab
Wild AMF	11 ± 0.2 c	5.7 ± 0.1 b	16.7 ± 0.2 c	33 ± 0.2 a	127 ± 3 b

AMF source strongly predicted plant growth (Appendix H), while colonization intensity was only weakly correlated ($R^2 = 0.14$, Tau = -4.89, $p<0.001$) and did not improve the biomass or height models (Chi-Square, $\chi 2 < 2.1$, $p > 0.078$). Plants with Farm and Wild AMF had the same average colonization intensity, yet plants with Farm AMF averaged 11 cm taller and 1 g heavier than those with Wild AMF (Table 7). Differences in plant height were negligible at the end of Phase 1, yet continued to increase over time (Figure 8).

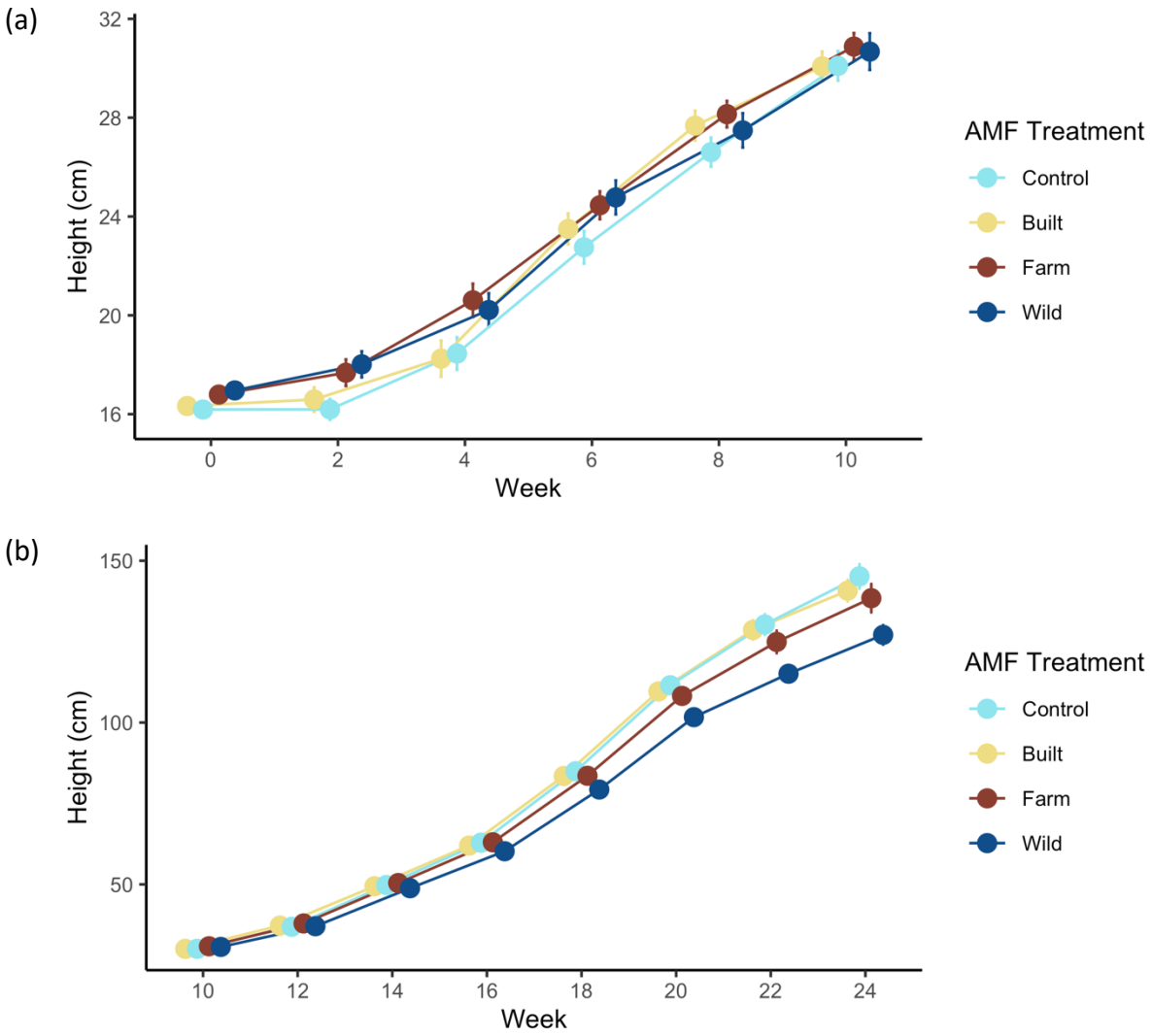


Figure 8: Heights of *R. idaeus* prepared with different AMF treatments, displayed as means ± standard errors from 40 replicates, averaged over stress treatments (a) during Phase 1 AMF treatments, and (b) after introduction of Phase 2 Stress treatments. Small differences in plant heights by treatments increased over time.

Plants which received *P. rubi, P. penetrans*, or both had similar height, total biomass, and leaf chlorophyll compared to plants with neither pest nor pathogen (Wald Chi-Square Test, $p > 0.05$). Plants which received nematodes had an average of 12% greater estimated dry root biomass than those which had no nematodes applied, but these differences may be an artifact of sample processing. Only plants with Wild AMF had no appreciable increase in root biomass

when nematodes were present (Table 8). The difference in root biomass between plants with and without nematodes was 22% larger in plants that received no AMF than it was for plants which received Farm AMF.

Table 8: Results of main effects contrast comparing root biomass (grams dry weight) of plants with and without nematodes applied. Bolded p-values highlight AMF treatments which had a significant root biomass response to nematodes.

AMF Treatment	**Root Biomass (g)**			**Stats**	
	Without	With	Difference	T	P-value
Control	5.9 ± 0.1	6.8 ± 0.2	1.0 ± 0.2	4.01	**<0.001**
Built	5.4 ± 0.1	6.2 ± 0.1	0.8 ± 0.2	3.60	**0.001**
Farm	5.8 ± 0.2	6.6 ± 0.1	0.8 ± 0.2	3.29	**0.003**
Wild	5.6 ± 0.2	5.9 ± 0.1	0.3 ± 0.2	1.22	0.233

Plant Nutrition

Shoot nutrient analyses revealed shoot nitrogen, phosphorous, and potassium content differed by $\leq$0.1% average difference between treatments. Phosphorous (P) was higher in plants with Farm or Wild AMF compared to those with Control or Built AMF (Tukey HSD, $p<0.001$; Table 9). AMF did not increase plant uptake of the other macronutrients under these greenhouse conditions (Appendix H).

Table 9: Plant nutrients measured in dried raspberry shoots. Plants were harvested following 24 weeks of growth. Data displayed are means ± standard errors from 40 replicates. Letters indicate significant differences following Tukey's pairwise comparisons.

AMF Treatment	N (%)	P (%)	K (%)
Control	0.9 ± 0.02 a	0.14 ± 0.004 b	1.19 ± 0.03 a
Built	0.9 ± 0.02 ab	0.15 ± 0.003 b	1.17 ± 0.02 a
Farm	0.8 ± 0.02 b	0.17 ± 0.004 a	1.26 ± 0.03 a
Wild	0.8 ± 0.02 ab	0.18 ± 0.004 a	1.19 ± 0.03 a

Stress Treatment Analyses

Plants in all stress treatments had similar heights and biomass compared to controls in the Summer Long Study, indicating we did not achieve levels sufficient to stunt plant growth (Appendix H). No *P. rubi* was isolated from the plant samples assessed. Plants receiving *P. penetrans* had 315-680 *P. penetrans*/g root at the time of harvest, with 44% lower densities in plants that also received *P. rubi* (Kruskal-Wallis test, $\chi 2 = 4.28$, $p = 0.038$), suggesting an interaction between these organisms (Figure 9).

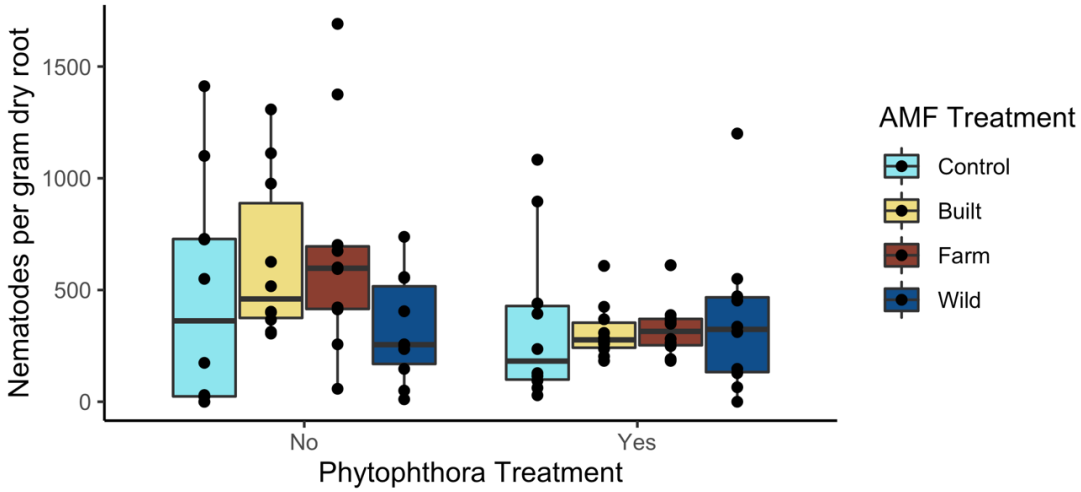
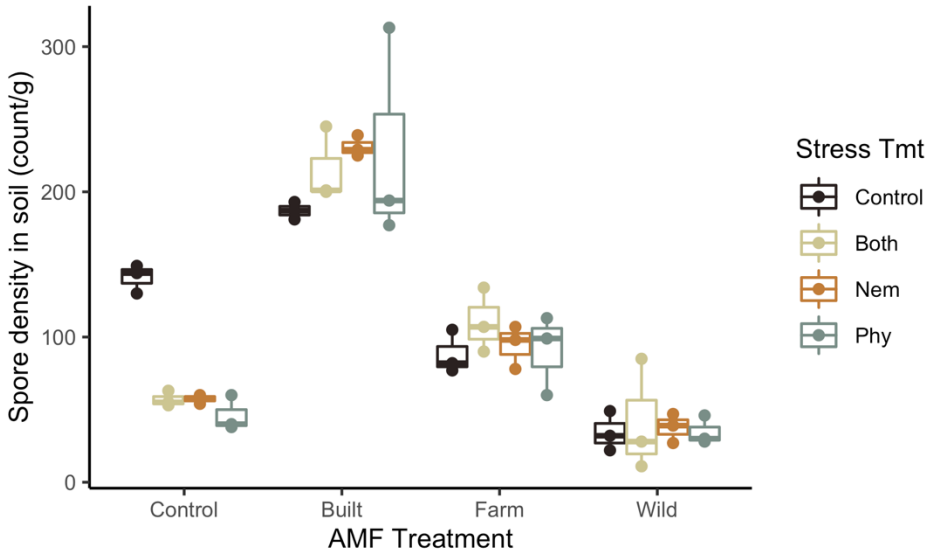


Figure 9: Density of nematodes within roots of *R. idaeus* at harvest (week 24). Boxplots show median and interquartile range of ten replicates per treatment combination. Root density of nematodes was 188 nematodes/gram root lower in plants inoculated with *P. rubi* compared to those without (Kruskal-Wallis test, $\chi 2 = 4.28$, $p = 0.038$).

Spore Extractions

The effect of pests/pathogens on AMF spore production was dependent on whether plants received early AMF inoculation. Within the AMF 'control' treatment, plants which received pest/pathogens had dramatically lower spore production than those which did not (Figure 10).

In contrast, within treatments that received early inoculation with AMF, there was not a significant difference in spore production (Figure 10).



AMF Treatment	Spore Density (count / g dry soil)			Stats	
	Without	With	Difference	T	P-value
Control	141 ± 6	53 ± 3	36 ± 15	2.5	**0.018**
Built	187 ± 3	225 ± 13	-15 ± 15	-1.1	0.288
Farm	88 ± 9	98 ± 7	-13 ± 15	-0.9	0.377
Wild	34 ± 8	38 ± 7	-5 ± 15	-0.35	0.733

Figure 10: Effect of exposure to *P. rubi or P. penetrans* on spore density in soils. Boxplots show median and interquartile range of three replicates per treatment combination. Data in table are means ± standard error from 3 replicates for 'without', and from nine replicates for 'with' (three replicates of each stress treatment).

3.3 Spring Short Study

Plants were more resilient to *P. rubi* when they were inoculated with Farm AMF, compared to those which received either Mykos AMF or no AMF. Survival to the end of the experiment was increased 300% by pre-inoculation with Farm soil, compared to controls (Figure 11). Though

Farm AMF reduced growth of unstressed plants, plants stressed by the pathogen *P. rubi* grew taller when colonized by Farm AMF compared to those with Mykos AMF or no AMF (Figure 11).

Of the plants which survived to the end of the experiment, those which were inoculated with Farm AMF were similar in size and height regardless of *P. rubi* exposure (Figure 12; Table 10). In contrast, plants prepared with no AMF or Mykos were stunted following exposure to *P. rubi*; averaging 6.3 g lower total biomass and 16.5 cm shorter than their unstressed counterparts (Table 10).

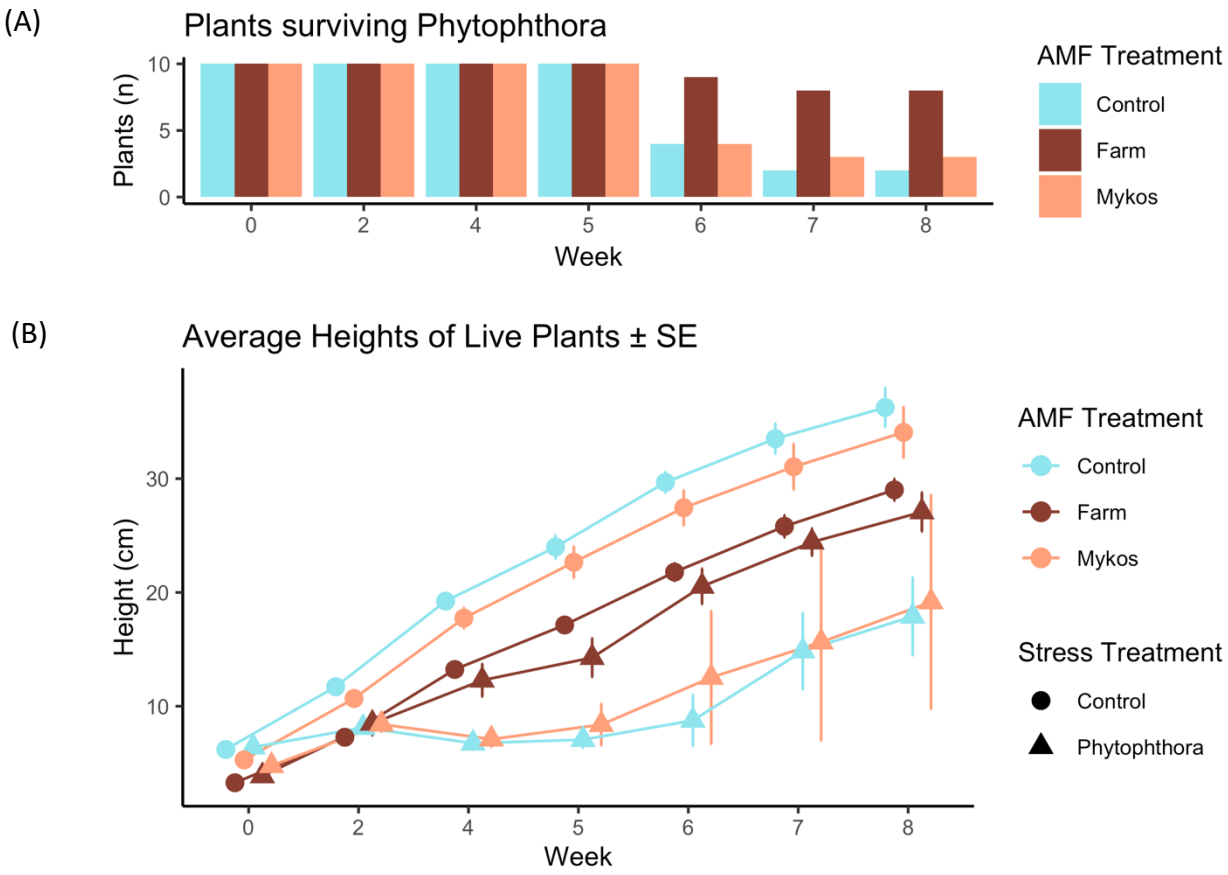


Figure 11: Survival and growth of *R. idaeus* prepared with different AMF treatments over the weeks following introduction of the stress treatments. (A) The number of plants surviving *Phytophthora rubi,* by AMF treatment. (B) Heights of live plants are displayed as means ± standard errors. The number of replicates varied; n=10 for all 'Control' stress treatment combinations, while n depended on survivorship for 'Phytophthora' treatments.

Table 10: Response of plants prepared with different AMF inoculum to *P. rubi*. Data presented are means ± standard errors; number of replicates (n) varies by treatment. For all treatments without *P. rubi,* n=10. For treatments which received *P. rubi*, n depends on survivorship at the end of the experiment: n = 2 for Control, n=3 for Mykos, and n=8 for Farm.

AMF Treatment	With *P. rubi*	Without *P. rubi*	Difference	T	P-value
Height (cm)					
Control	18 ± 2 c	36 ± 2 a	– 18 ± 4.9	3.8	**0.001**
Mykos	19 ± 5 c	34 ± 2 ab	– 14 ± 4.2	3.6	**0.001**
Farm	27 ± 2 bc	29 ± 1 abc	– 2 ± 3.0	0.7	0.518
Shoot Biomass (GDW)					
Control	0.9 ± 0.1 d	3.9 ± 0.1 a	– 3.0 ± 0.4	7.6	**<0.001**
Mykos	1.5 ± 0.5 cd	3.5 ± 0.2 b	– 2.0 ± 0.3	5.8	**<0.001**
Farm	2.1 ± 0.1 c	2.2 ± 0.1 cb	– 0.1 ± 0.2	0.5	0.616
Root Biomass (GDW)					
Control	0.4 ± 0.1 b	6.1 ± 1.5 a	– 5.7 ± 2.0	2.8	**0.008**
Mykos	2.3 ± 0.6 ab	4.2 ± 0.4 ab	– 1.9 ± 1.2	1.1	0.267
Farm	2.6 ± 0.4 ab	1.8 ± 0.2 b	+ 0.8 ± 1.9	0.7	0.499

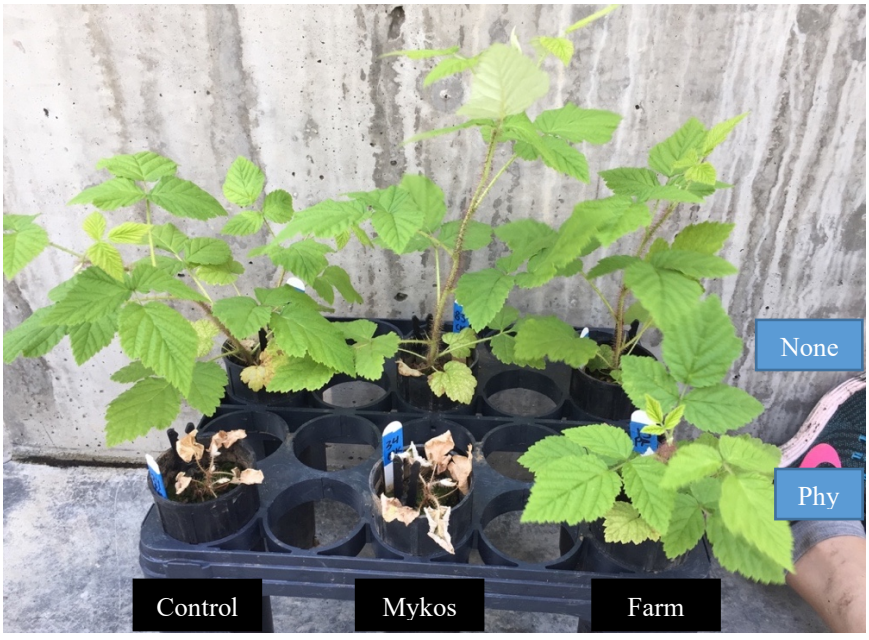


Figure 12: Typical plant condition for each treatment, photographed 8 weeks following introduction of *P. rubi.* Plants in each column received the same AMF treatment (black labels). Rows contain plants in the same stress treatment (blue labels). Those with Farm AMF tended to grow well regardless of *P. rubi* exposure.

4. Discussion

Farm soil inoculum improved tolerance to *Phytophthora rubi*

Inoculation of red raspberry (*R. idaeus*) with appropriate microbial communities prior to out-planting into fields could promote successful establishment in risk-prone soils. In our Spring Short Study, inoculation of young *R. idaeus* cv. Meeker with a mature soil community improved subsequent disease resistance of transplanted plants, though inoculation with a commercial AMF product did not (Table 10). Plants inoculated with Farm soil not only survived *P. rubi* exposure 300% more than uninoculated controls (Figure 10), but the surviving plants were also taller and more robust than uninoculated controls (Table 10). Although we did not ascertain the specific mechanisms by which beneficial biota within the farm soil, such as AMF, could have mitigated damage by *P. rubi*, both direct and indirect mechanisms have been well documented in other plant-pathosystems. Direct interactions between the soil microbial community and *P. rubi* that could have limited detrimental impacts to plants include competition between AMF and the pathogen for infection sites on roots (Vigo et al. 2001), and production of compounds antagonistic to pathogen growth by soil microbes such as plant-growth promoting bacteria (Azcón-Aguilar and Barea 1997). Indirect effects of the soil microbial community which are also known to impact pathogen success include changes to root exudation (Norman and Hooker 2000), and induction of plant's systemic defenses by AMF infection (Pozo et al. 2002).

While these results are promising, survival at this stage is only one indicator of future plant success. Even if AMF colonization can improve tolerance of *Phytophthora* infection, infected plants can fail later in the season or in later years– through a lack of bud break on

floricanes, or the collapse of fruiting laterals before or during fruiting (CABI and EPPO 1996). That said, as suggested already, older plants may be more capable of resisting *P. rubi*, so increases in establishment success may constitute an important step toward sustainable crop productivity.

Specific temporal and environmental conditions have a strong influence on plant succeptibility to *P. rubi*. In our studies, infection only occurred in the Spring Short Study where we inoculated younger plants, maintained cooler temperatures, and sustained saturation. A lack of plant biomass response to *P. rubi* in the Summer Long Study (Table H1) indicated failure of *P. rubi* to cause disease, likely due to high greenhouse temperatures directly following introduction of the pathogen, combined with the age of the plants. Temperatures exceeding 30°C (86°F) impair growth and infectivity of *P. rubi* (Duncan 1985). During the Summer Long Study, greenhouse temperatures exceeded that threshold for 1-3 hours every day in the week following inoculation, and spiked over 38°C (100°F) three times during that week (Appendix A). Use of older plants probably further reduced their vulnerability to the pathogen – while plants in our Summer Long Study were over 10 weeks old at the time of pathogen introduction, most greenhouse experiments demonstrating mortality of *R. idaeus* due to *P. rubi* infection have introduced the pathogen to young plants (Gigot et al. 2013; Wilcox et al. 1993). Although the mechanism is unknown, Raftoyannis and Dick (2006) observed higher rates of *Phytophthora* zoospore encystment – the loss of motility and structural changes that precede germination and infection by the pathogen – on young plants than older plants.

The benefits of AMF colonization were only observed under conditions favorable to pathogen growth, leading us to conclude that that benefits of AMF colonization of raspberry

change across environmental and temporal gradients. In the absence of stressors, AMF colonization led to moderate reductions in plant heights and biomass, indicating that AMF may be parasitic on raspberries under those conditions. In the Summer Long Study, where no plants had responses to the pest/pathogens, higher levels of AMF colonization were coupled with an 8-14% reduction in plant biomass. Similarly, in our Spring Short Study, uninoculated plants grew almost twice as tall as those with AMF in the absence of stressors, but with a pathogen present this pattern was reversed (Figure 10). This is consistent with other studies that find there is a tradeoff in the plant-AMF symbioses; even if costs to plants exceed benefits in the absence of stressors, the symbiosis might offer substantial benefits in the presence of stressors (Jacott et al. 2017; Johnson 2010). This highlights the importance for research into the potential roles of AMF in sustainable crop management to incorporate stressors that host plants would encounter in field conditions.

Raspberry farm soil outperformed other AMF sources

In contrast with our expectations, we found that beneficial AMF persist under commercial raspberry cultivation. Farm soil harbored AMF propagules at a similar density (Table 4) and infectivity (Table 6) as Wild soil – both colonized 91% of roots by 24 weeks. This was unexpected since fumigation and fertilization, common practices in commercial raspberry production, have long been associated with diminished abundance, species diversity, and infectivity of AMF (Belay et al. 2015; Dangi et al. 2015; Verbruggen et al. 2012; Jansa et al. 2009). That said, a review by Ryan and Graham (2018) concluded that AMF literature may

overstate the magnitude of detrimental impacts to AMF communities by common agricultural practices, which may be the case for WA raspberry production.

The abundance and viability of AMF in this farm soil may be explained in several ways. First, the specific soil fumigant used at this site, Telone C-35, is less detrimental to AMF than many other fumigants (Dangi et al. 2015; De Cal et al. 2005; Ibekwe et al. 2001). Second, there may be sufficient dispersal of AMF from adjacent fields and wild lands to replenish management-derived reductions in abundance. Although AMF spores are formed underground in roots and in soil, they can be dispersed by animals (Vernes and Dunn 2009, Lekberg et al. 2011) and are commonly dispersed by wind, especially during dry seasons (Warner et al. 1987, Allen et al. 1989). Third, AMF communities shift in response to land use, and it is possible that the AMF present in the Farm soil represent local adaptation to that management regime (Pellegrino et al. 2020), thus colonization may be less inhibited by nutrient levels present in those systems. Although AMF communities and resulting colonization are likely to differ between and within farms, as observed in our Preliminary Observational Study (Section 3.1), it is possible that other raspberry fields undergoing similar management could have sufficient AMF to colonize their plants.

Plants derived greater net benefits from local Farm soil than any other AMF source tested. In our Summer Long Study, inoculation with Farm or Wild AMF resulted in 15% higher levels of colonization (Table 6) and 21% higher shoot phosphorous than plants in Control or Built treatments (Table 9). While these benefits were offset by a large height reduction for plants with Wild AMF (Table 7), heights of those with Farm AMF were not significantly reduced (Table 7). High colonization by the Wild and Farm AMF in the Summer Long Study may have

been supported by the synergistic effects of bacteria endemic to those AMF communities (Artursson et al. 2006), the benefits of which can be taxa-specific (Turrini et al. 2018). These AMF-associated bacteria can improve germination and growth of AMF, colonization by AMF, and even increase plant benefits from colonization, although the mechanisms underlying these effects are not well understood (Artursson et al. 2006). Although all plants in our Summer Long Study received the same microbial wash, only Wild and Farm inoculum contributed intact soil communities to that wash, therefore small microbes that act synergistically with the AMF taxa in those treatments may have been present while those naturally associated with the AMF in the Built inoculum were lacking.

Similarly, in the Spring Short Study, inoculation with Farm soil offered dramatic benefits to stressed plants, while the commercial Mykos AMF inoculum did not (Section 3.3). Such heightened benefits from Farm inoculum might be explained by local adaptation of AMF available in the Farm soil to raspberry plants, or by the presence of other microbiota in the Farm soil, which were absent in the Mykos treatment. Various soil microbes have the potential to contribute to pathogen suppression and plant resilience through direct effects (Azcón-Aguilar and Barea 1997) and through synergism with AMF (Artursson et al. 2006). In agreement with the findings of other studies primarily focused on natural systems (Emam 2016, Rúa et al. 2016, Antunes et al. 2011), these results suggest that local adaptation or species specificity of the Farm soil microbial community may improve raspberry plant responses to inoculation.

The importance of timing

Availability of beneficial biota in Farm soil does not preclude the utility of inoculating plants prior to planting in the field. The Farm soil inoculum used in our studies was obtained from an 18-month old raspberry stand, and the microbial community there could be very different from that present at the time of crop planting. Techniques used to prepare a field, such as tillage, can substantially reduce AMF abundance in the short term even if those AMF populations recover over time (Rasmann *et al.* 2009). Additional research would be needed to determine if similar benefits could be derived from the microbial community present following the processes that growers use to prepare fields before planting *R. idaeus*.

Inoculation of plants with beneficial microbial communities before field exposure to potential pests and pathogens may maximize potential benefits to raspberry crops. Early inoculation with AMF can have lasting effects, and the importance of timing has been demonstrated both in our experiments and in prior studies (Emam 2016; Forge et al. 2001; Talavera et al. 2001). For example, Forge et al. (2001) found that AMF inoculation improved resilience to parasitic nematodes only if the AMF were introduced before the pests. In our Summer Long Study, all plants received additional airborne inoculum from the open venting greenhouse, similar to conditions that might be found in fields, leading to all plants, including control treatments, becoming colonized with AMF by the end of the study (Table 6). However, biomass differences between AMF treatments continued to increase over the course of the experiment, suggesting that early inoculation had a greater influence than subsequent inoculation (Figure 8).

Stress treatments had a greater effect on AMF spore density when AMF arrived later, suggesting that early AMF success (infectivity or reproduction) might be negatively affected by root pathogens. Within the Summer Long Study 'Control' AMF treatment, which had minimal colonization by AMF contaminants at the time of pest/pathogen introduction (Table B1), soil density of AMF spores was 36 spores/GDW higher in the stress-control pots, and colonization was 26% higher for plants in stress-control treatments (Table E1). In contrast, all other AMF treatments had fairly consistent spore densities across all stress treatments, less than 10% difference (Table E1). Reduced AMF colonization and spore production when pest/pathogens were already present could be to increased competition in the soil, either for nutrients or infection sites on the roots, or because plants' defense systems were upregulated in response to the biotic stressors such that AMF were unable to colonize the roots to the same extent that they otherwise could have (De Souza et al. 2016, Azcón-Aguilar and Barea 1997).

Mycorrhizae formation regardless of high soil phosphorous

A primary argument against the utility of AMF in production agriculture is their sensitivity to high soil nutrients, yet we have demonstrated *R. idaeus* is capable of forming mycorrhizae even under high soil phosphorous conditions common to commercial raspberry farms. Both our studies utilized soils with 280 – 351 ppm phosphorous, and despite this AMF inoculation led to high colonization (60-100%) across all our AMF treatments. While this contrasts with many studies demonstrating that high phosphorous inhibits the formation of mycorrhizae (Ryan & Graham 2002; Thomson et al. 1986), others have demonstrated AMF populations persist in high phosphorous soils, though the community composition changes (Van

Geel et al. 2015; Verbruggen et al. 2012), presumably toward AMF tolerant of those conditions. In our experiments, both Farm and Wild AMF were sourced from P-rich soils (Table 2), and local adaptation to those conditions may supported colonization capacity. Successful colonization occurring in all AMF treatments could also be due to the plant cultivar involved as much as the AMF. The capacity of plants to exclude AMF under sufficient nutrient conditions, or to exclude uncooperative AMF, varies by plant taxa (Johnson 2010) and cultivar, and could be impaired in domesticated plants (Porter and Sachs 2020, Xing et al. 2012).

Pest/Pathogens may shift AMF community composition

Though pest/pathogens introduced after AMF community establishment had a negligible effect on total spore density, the AMF community composition was altered. We were able to quantify this change within the Summer Long Study 'Built' treatment, where plants receiving parasitic nematodes had a significantly higher density of *D. heterogama* spores at the end of the experiment, and somewhat lower densities of *C. etunicatum* spores (Figure I1). Although there is limited research into such interactions between plant pests/pathogens and AMF community structure, the mechanism could fall into two categories: either direct effects of the pest on particular fungal species, or indirect effects of changes to the plant-fungal relationship. If the effects (direct or indirect) of nematode pressure are unequal across AMF taxa, that could lead to a shift in community composition, such as we observed. The results of Brito et al. (2018) highlighted unequal effects of nematodes on AMF spore production – between six AMF species cultivated separately on maize (*Zea mays* L.), the they found

increased spore production by *Rhizophagus clarus* in the presence of plant-parasitic nematodes (*Pratylenchus brachyurus)*, but no significant changes for other AMF species.

Nematodes did not challenge plants, and might be inhibited by *P. rubi*

Nematodes did not impair plant growth at our treatment densities. Our observed lack of plant biomass response is consistent with the results of Gigot et al. (2013) who also applied *P. penetrans* at the action threshold of 1 nematode/gram soil, and observed no plant biomass response. Higher densities of nematodes have been observed to significantly impair *R. idaeus* establishment and productivity, as demonstrated in the experiment by Zasada et al. (2015) which had a treatment containing 100x as many nematodes per gram soil, and 16x as many nematodes per gram root, compared with the final densities in our study (Table F2).

Against our expectations, plants which received nematodes had significantly more root biomass than those which did not receive nematodes, regardless of AMF treatment (Table 8). While this could be an artifact of estimating dry biomass from wet weights of roots which were sent away for analysis, the increase in root biomass was not observed for one AMF treatment (Wild), and so this is unlikely. While such a positive plant biomass response to herbivorous nematodes has not been documented for raspberry (*R. idaeus*), several studies demonstrated a positive response by grasses (Bardgett et al. 1999, de la Pena et al. 2005, Gebremikael et al. 2016). For example, biomass of beach grass (*Ammophilia brevigulata*), was increased by co-inoculation with AMF and similar densities of nematodes (*P. penetrans*) as used in our study (de la Pena et al. 2005). Plausible mechanisms for such positive biomass effects include soil community-level responses in which herbivory stimulates the release of C-rich root exudates

into the soil, which increases soil microbial activity and nutrient availability for plant growth (Bardgett et al. 1999, Gebremikael et al. 2016) and can lead to stimulation of plant growth hormones by these soil biota (Mao et al. 2006). Other biological explanations could include the introduction of plant-beneficial microbes along with the nematode extracts (Adesemoye et al. 2008), or a small nutrient effect from the additional organisms available to the soil food web for decomposition. Whether the increase in root biomass was a real treatment effect or an artifact cannot be determined, as the amount of roots estimated was largest for plants in the nematode treatment, and every plant in the nematode treatment had a large volume of roots estimated, thus confounding the treatment effect with the effects of estimation. Approximately half of the total root mass for each plant with nematodes was sent for analysis, and the weight estimated using a linear regression ($R^2 = 0.78$), which could not include other features that would have influenced the relationship between wet and dry weight such as the proportion of fine vs woody roots.

Interestingly, *P. penetrans* may be inhibited by *P. rubi.* Presence of *P. rubi* significantly reduced density of nematodes both in plant roots and soil (Figure 9). This could be due to direct interactions between *P. rubi* and *P. penetrans* in the soil, or due to indirect effects such as altered root exudation or upregulation of plant defenses in response to *P. rubi*. Although we did not observe successful infection by *P. rubi,* plant defenses primed by early detection of pathogens can improve resilience to subsequent invaders. Many species of *Phytophthora* release elicitors that can be recognized by the host plant even before infection begins, such as cell wall glucans released during the germination of encysted zoospores (Waldmueller et al. 1992, Taylor 2002). Recognition of such elicitors can prime defense pathways important for

plant's resistance to infection by a variety of other organisms, including nematodes (Keller et al. 1999, Cooper et al. 2005, Pieterse et al. 2014, Schouteden et al. 2015). Beneficial biota, such as AMF, also trigger plant defense pathways during infection, which leads to a similar induction of systemic resistance to pests and pathogens (Pozo et al. 2002, Schouteden et al. 2015). The observed reduction in nematodes was greater for those plants with Farm or Built AMF, while those with Wild AMF had low nematode numbers regardless of whether they were exposed to *P. rubi* (Figure 9). This could indicate that the mechanism by which *P. rubi* led to reduced nematodes is not a direct interaction between *P. rubi* and *P. penetrans*, but rather an indirect effect which could be triggered by other species, such as AMF. Our results contrast with an earlier study by Gigot et al. (2013) which found the opposite; the highest densities of *P. penetrans* were observed in root tissues when plants were inoculated with the highest densities of *P. rubi*. The difference in observed results could be an effect of timing, plant age, or inoculum densities. In our study the plants were exposed to *P. rubi* before the first nematode application, plants were weeks older, pest and pathogen applications differed, and environmental conditions were less conducive to *P. rubi* growth than in Gigot et al. (2013).

5. Conclusion

In conclusion, our data shows that 'Meeker' *R. idaeus* associate readily with AMF under high-phosphate conditions common in managed fields, and that viable communities of AMF can persist in commercial production fields. These data cast doubt on the utility of introducing foreign AMF to fields which may already have abundant and more supportive endogenous AMF present. We have also demonstrated for the first time that *R. idaeus* inoculated with a healthy soil community can have enhanced resistance or tolerance to disease caused by *P. rubi*. Furthermore, both studies highlight that the source of AMF matters; AMF naturally occurring in an agricultural soil resulted in higher colonization and improved survivorship of the plants which they colonized compared to AMF from either constructed or commercial inoculum sources.

While this study provides only a snapshot of the possibilities based upon the AMF community available on one farm, we believe there is potential to harness the benefits of that symbioses to improve raspberry tolerance to stressors. These results suggest that the slight tradeoff in plant biomass resulting from partnership with AMF is offset by the benefits derived when exposed to root pathogens, such as *P. rubi*. For growers seeking to reduce dependence on chemical pathogen control, there is clear potential for the inoculation of tissue culture plantlets with beneficial soil biota to improve survival following transplanting into production fields. Further research should be directed to investigate how these findings might be altered by higher levels of nitrogen fertilization, whether similar improvements in plant-soil-feedback will be observed in field conditions over longer time spans.

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Appendix A: Environmental Data

Throughout the Summer Long Study, Environmental data (temperature and humidity) was tracked using an Onset® HOBO data logger (Bourne, MA), located at soil surface height between plants of Block 2. Light data was collected around noon every other week using a LI-250A light meter (LI-COR Biosciences; Lincoln, NE), recorded as a 10 second average across each block.

Light

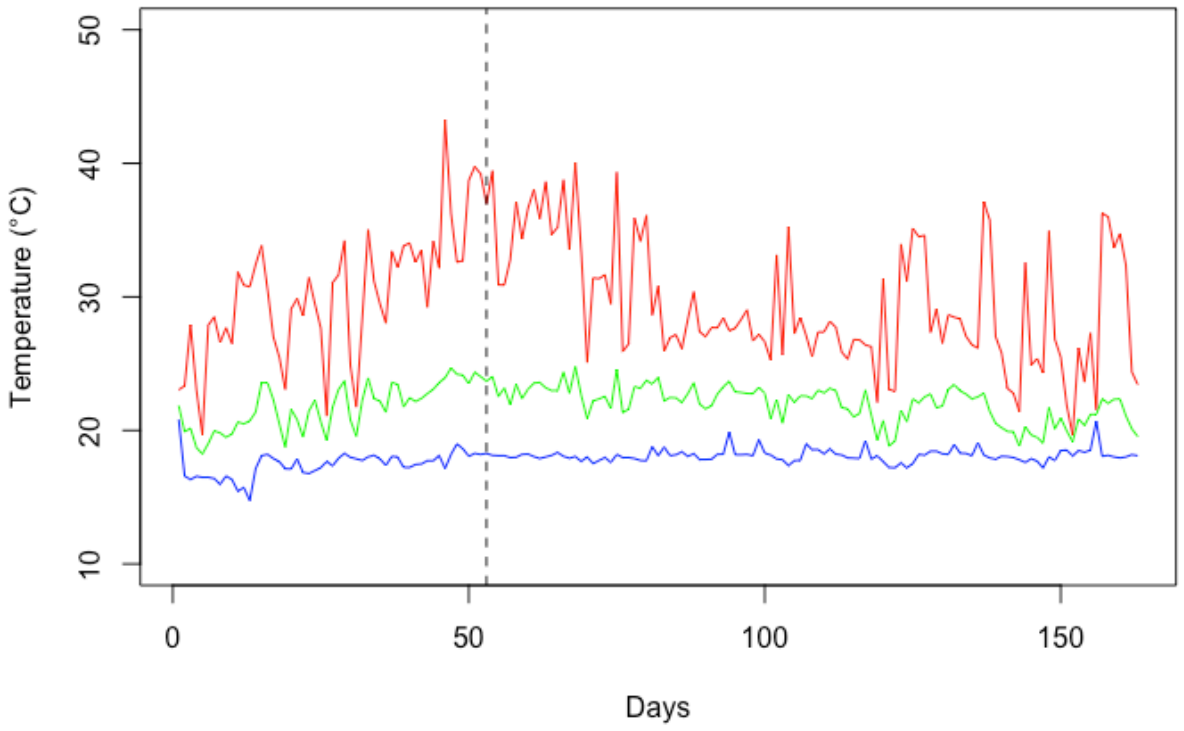
Average light was highest in May and June (Table A1), when the greenhouse shade was not drawn. After we began regularly closing the greenhouse shade to reduce heat in the greenhouse, plants received less afternoon light.

Table A1: Light levels experienced by plants in each block around noon. Data presented are mean light levels recorded at each block, averaged by month.

	Average Light (mmols/second)					
Block	April	May	June	July	August	September
1	314.9	543.5	295.8	286.0	107.4	213.7
2	118.8	450.5	400.8	239.5	95.8	200.6
3	262.5	389.3	393.0	245.8	101.2	243.0
4	232.7	514.5	391.1	293.2	124.8	238.1
5	233.8	437.7	403.4	228.5	119.8	280.9
6	233.8	437.7	448.8	151.4	95.5	133.2
7	232.7	514.5	302.8	216.7	103.7	126.0
8	262.5	389.3	389.7	317.3	113.8	156.9
9	118.8	450.5	282.9	250.0	111.3	178.9
10	314.9	543.5	357.3	191.2	106.5	159.8

Temperature Data

Maximum temperatures exceeded the ideal growing range for raspberry (70 – 75°F) on 145 days of the experiment, topping 100°F on 10 days. That said, the daily average temperatures were within the "ideal growing range" on 92% of days (Figure A1).



Month	Average Temp (°C)	Average Daily High (°C)	Average Daily Low (°C)
April	19.9	26.7	16.5
May	21.9	29.9	17.6
June	23.2	35.0	18.1
July	22.6	29.0	18.2
August	21.9	27.9	18.2
September	20.6	27.7	18.1

Figure A1: Greenhouse temperature trends for the Summer Long Study (2019). The plot shows trends in daily average (green), highs (red) and low (blue) temperatures over the course of the experiment, with days on the x-axis. Vertical dashed line marks the date at which plants were introduced to their Phase 2 Stress treatments. The table further summarizes these trends by month (means).

Appendix B: Midpoint Harvest

Three plants in each Phase 1 AMF treatment of the Summer Long Study harvested at the same time that all other plants would be repotted into their Phase 2 Stress treatments (Week 10). These plants had all grown together in Block 11 (between Blocks 1 and 6).

Small differences in growth of this small subset of plants were not significant; plants with Wild AMF were just slightly taller and larger than those in other treatments (Table B1), which is consistent with the trends observed outside this block at week 10 (Figure 8).

Table B1: Dry weight, heights, and leaf chlorophyll of three 10-week old 'Meeker' raspberry plants from each treatment, harvested before the rest of the plants were introduced to Phase II treatments. Phase I treatment is shown in the left column. Data displayed are means ± standard errors from 3 replicates.

AMF Treatment	Shoot biomass (g/plant)	Root biomass (g/plant)	Total biomass (g/plant)	Leaf chlorophyll (SPAD)	Height (cm)	Nodes (count)
Control	2.2 ± 0.2	0.6 ± 0.0	3.6 ± 0.2	22 ± 0.5	31 ± 0.6	12 ± 0.7
Built	2.2 ± 0.2	0.6 ± 0.0	3.5 ± 0.2	20 ± 0.6	30 ± 1.1	11 ± 0.7
Farm	2.2 ± 0.2	0.6 ± 0.1	3.4 ± 0.3	21 ± 0.9	30 ± 0.8	11 ± 1.2
Wild	2.4 ± 0.3	0.8 ± 0.1	4.1 ± 0.5	20 ± 1.2	32 ± 0.7	11 ± 0
ANOVA	$F_{3,8}$ = 0.3, p=0.8	$F_{3,8}$ =1.5, p=0.3	$F_{3,8}$ = 1.0, p=0.5	$F_{3,8}$ = 0.8, p=0.5	$F_{3,8}$ = 1.9, p= 0.2	$F_{3,8}$ = 0.13, p=0.9

Plants in all treatments had higher total colonization at the end of 24 weeks (Table 6) than observed in the small subset harvested at Week 10 (Table B2). Colonization trends at Week 10 were also much different than would be observed by the end of the main experiment (Table 6). Specifically, though Farm and Wild AMF would result in similar total colonization by week 24 (Table 6), this harvest indicated those with Wild AMF had only about half the root colonization as those with Farm AMF (Table B2). Additionally, plants with Built AMF had the

highest colonization rates of any group (Table B2), though by the end they would have only about half the colonization rate observed in plants of the Farm and Wild treatments.

Table B2: Colonization observed in fine roots of 10-week old 'Meeker' raspberry plants. Data displayed are means ± standard errors from 3 replicates. Approximately 72 intersections were assessed for each plant.

AMF Treatment	Vesicles (%)	Arbuscules (%)	Both V & A (%)	Hyphae (%)	Total % AM
Control	0	6 ± 3	0	2 ± 0	8 ± 3
Built	2 ± 0	39 ± 4	0	19 ± 2	59 ± 6
Farm	2 ± 2	22 ± 1	1 ± 1	21 ± 5	44 ± 5
Wild	2 ± 1	5 ± 2	0	22 ± 2	29 ± 2

Appendix C: Growth Measures

Results of the destructive harvest for the Summer Long Study. All plants in the study survived to the end of the 24-week experiment, regardless of stress treatments.

Table C1: Dry weight, heights, and leaf chlorophyll of 24-week old 'Meeker' raspberry plants. Plants received inoculation with AMF from sources in the first column on day 0, and received the stress treatments in the second column at week 10. Data displayed are means ± standard errors from 10 replicates.

Treatment combination (n=10)		Shoot biomass (g/plant)	Root biomass (g/plant)	Total biomass (g/plant)	Leaf chlorophyll (SPAD)	Height (cm)	Nodes
Control	Control	12.9 ± 0.6	6.0 ± 0.2	19.0 ± 0.8	32 ± 0.6	152 ± 9	41 ± 1
	Pratylenchus penetrans	12.1 ± 0.5	7.0 ± 0.3	19.1 ± 0.8	31 ± 0.5	134 ± 11	41 ± 2
	Phytophthora rubi	12.4 ± 0.3	5.7 ± 0.2	18.1 ± 0.4	32 ± 0.3	145 ± 4	42 ± 2
	Both	13.0 ± 0.5	6.7 ± 0.2	19.8 ± 0.6	30 ± 0.5	149 ± 4	43 ± 1
Built	Control	12.2 ± 0.4	5.8 ± 0.3	18.1 ± 0.6	32 ± 0.4	140 ± 9	40 ± 2
	Pratylenchus penetrans	11.8 ± 0.4	6.5 ± 0.2	18.3 ± 0.5	33 ± 0.4	134 ± 7	42 ± 2
	Phytophthora rubi	11.9 ± 0.3	5.7 ± 0.2	17.6 ± 0.3	33 ± 0.3	144 ± 6	38 ± 2
	Both	12.1 ± 0.2	6.7 ± 0.2	18.8 ± 0.4	33 ± 0.2	145 ± 4	41 ± 1
Farm	Control	12.2 ± 0.5	5.5 ± 0.1	17.7 ± 0.5	32 ± 0.5	139 ± 7	40 ± 2
	Pratylenchus penetrans	11.8 ± 0.6	6.1 ± 0.2	17.9 ± 0.7	33 ± 0.6	147 ± 8	39 ± 2
	Phytophthora rubi	11.1 ± 0.4	5.3 ± 0.1	16.4 ± 0.4	34 ± 0.4	122 ± 12	42 ± 1
	Both	12.0 ± 0.5	6.2 ± 0.2	18.3 ± 0.6	31 ± 0.5	145 ± 7	42 ± 1
Wild	Control	11.0 ± 0.3	5.8 ± 0.2	16.8 ± 0.4	34 ± 0.3	122 ± 6	38 ± 1
	Pratylenchus penetrans	10.5 ± 0.2	5.8 ± 0.2	16.3 ± 0.3	34 ± 0.2	127 ± 3	38 ± 1
	Phytophthora rubi	11.3 ± 0.3	5.3 ± 0.3	16.6 ± 0.5	34 ± 0.3	132 ± 4	38 ± 1
	Both	11.1 ± 0.5	5.9 ± 0.2	17.1 ± 0.5	32 ± 0.5	127 ± 9	37 ± 2

Appendix D: Shoot Nutrient Data

Shoot nutrient content differed by an average of under 0.1% between treatments. When nematodes were applied, potassium (K) content was modestly but significantly reduced for plants in the Control and Farm AMF treatments (Main Effects Contrast, $p < 0.05$).

Table D1: Shoot nutrient content of 24-week old *R. idaeus* cv. Meeker. Plants received early inoculation with AMF treatments in the first column, and received the stress treatments in the second column at week 10. Data displayed are means ± standard errors from 10 replicates.

Treatment combination		Nitrogen (%)	Phosphorous (%)	Potassium (%)	Magnesium (%)	Copper (ppm)
Control	Control	0.9 ± 0.04	0.1 ± 0.01	1.2 ± 0.05	0.3 ± 0.01	1.8 ± 0.23
	Pratylenchus penetrans	0.9 ± 0.03	0.1 ± 0.01	1.2 ± 0.04	0.3 ± 0.02	1.8 ± 0.18
	Phytophthora rubi	0.9 ± 0.03	0.2 ± 0.01	1.3 ± 0.04	0.3 ± 0.01	2.1 ± 0.28
	Both	0.9 ± 0.03	0.1 ± 0.01	1.1 ± 0.04	0.3 ± 0.02	1.2 ± 0.15
Farm	Control	0.8 ± 0.03	0.2 ± 0.01	1.3 ± 0.07	0.3 ± 0.02	2.6 ± 0.32
	Pratylenchus penetrans	0.9 ± 0.05	0.2 ± 0.01	1.2 ± 0.04	0.3 ± 0.02	2.4 ± 0.27
	Phytophthora rubi	0.9 ± 0.04	0.2 ± 0.01	1.3 ± 0.04	0.4 ± 0.01	2.8 ± 0.20
	Both	0.8 ± 0.04	0.2 ± 0.01	1.2 ± 0.05	0.3 ± 0.01	2.4 ± 0.17
Wild	Control	0.9 ± 0.05	0.1 ± 0.01	1.1 ± 0.03	0.3 ± 0.01	1.8 ± 0.13
	Pratylenchus penetrans	0.8 ± 0.03	0.1 ± 0.01	1.1 ± 0.04	0.3 ± 0.01	1.9 ± 0.17
	Phytophthora rubi	0.9 ± 0.02	0.1 ± 0.00	1.2 ± 0.04	0.3 ± 0.01	2.0 ± 0.06
	Both	0.9 ± 0.03	0.1 ± 0.01	1.2 ± 0.04	0.3 ± 0.01	1.9 ± 0.09
Built	Control	0.8 ± 0.03	0.2 ± 0.01	1.2 ± 0.04	0.3 ± 0.01	2.9 ± 0.17
	Pratylenchus penetrans	0.8 ± 0.04	0.2 ± 0.01	1.1 ± 0.05	0.3 ± 0.02	2.9 ± 0.15
	Phytophthora rubi	0.9 ± 0.05	0.2 ± 0.01	1.2 ± 0.07	0.3 ± 0.02	2.8 ± 0.26
	Both	0.9 ± 0.05	0.2 ± 0.01	1.2 ± 0.03	0.3 ± 0.01	2.9 ± 0.28

Appendix E: Colonization

Colonization was assessed for 7 of 10 replicates in each treatment combination of the Summer Long Study (Table E1). Plants with Built AMF tended to have more arbuscules, while those with Farm and Wild AMF exhibited the highest number of vesicles.

Table E1: Colonization observed in fine roots of 24-week old *R. idaeus* cv. Meeker plants. Plants received early inoculation with AMF treatments in the first column, and received the stress treatments in the second column at week 10. Data displayed are means ± standard errors from 7 replicates. Approximately 72 intersections were assessed for each plant.

Treatment combination		Vesicles (%)	Arbuscules (%)	Both V & A (%)	Hyphae (%)	Total % AM
Control	Control	5 ± 2.3	13 ± 1.3	0 ± 0.1	45 ± 4.1	62 ± 4.6
	Pratylenchus penetrans	4 ± 1.1	7 ± 1.1	0 ± 0.1	36 ± 3.9	46 ± 5.5
	Phytophthora rubi	8 ± 2.1	9 ± 2.3	2 ± 0.7	40 ± 5.2	57 ± 7.7
	Both	6 ± 1.5	8 ± 0.8	4 ± 0.8	41 ± 3.3	56 ± 4.4
Built	Control	3 ± 0.5	19 ± 1.5	0 ± 0.2	55 ± 1.9	76 ± 1.5
	Pratylenchus penetrans	3 ± 0.6	17 ± 0.9	0 ± 0.1	59 ± 1.3	80 ± 1.5
	Phytophthora rubi	2 ± 0.3	15 ± 1.5	1 ± 0.4	61 ± 2.2	79 ± 1.6
	Both	3 ± 0.6	18 ± 2.1	3 ± 0.4	46 ± 2.2	67 ± 2
Farm	Control	16 ± 2	9 ± 1	1 ± 0.3	56 ± 1.8	85 ± 1.7
	Pratylenchus penetrans	18 ± 0.8	13 ± 1.1	1 ± 0.1	56 ± 2.1	90 ± 1.4
	Phytophthora rubi	34 ± 2.7	11 ± 1	1 ± 0.2	48 ± 2	96 ± 1.2
	Both	19 ± 1.4	11 ± 1.2	2 ± 0.6	61 ± 1.9	92 ± 1.5
Wild	Control	20 ± 3.6	11 ± 2	0 ± 0.2	63 ± 4.4	96 ± 1.2
	Pratylenchus penetrans	12 ± 1.2	6 ± 0.6	0 ± 0.1	68 ± 1.8	87 ± 1
	Phytophthora rubi	20 ± 1.9	6 ± 1.1	4 ± 0.8	64 ± 3	94 ± 0.9
	Both	7 ± 1.3	10 ± 2.7	3 ± 0.5	68 ± 2.1	86 ± 1.2

Appendix F: Nematodes

Nematodes were quantified prior to application (Weeks 10 – 15) by counting on a McMaster slide. At the end of the experiment (Week 24), nematode densities were quantified both within roots and within soil.

Applications

In the Summer Long Study, nematode treatments were sourced from harvested roots of infected mature plants. We applied nematodes to plants as quickly as we could extract them from source roots, which was approximately biweekly. Over the course of 40 days, we applied approximately 1558 ± 121 nematodes to each plant in the nematode treatment (Table F1).

Date	Nematodes / plant
6/13	95 ± 6
6/17	39 ± 4
6/20	38 ± 10
6/24	59 ± 11
6/27	4 ± 2
7/1	3 ± 2
7/4	153 ± 18
7/8	185 ± 21
7/11	305 ± 21
7/18	126 ± 14
7/22	551 ± 12
Total	**1558 ± 121**

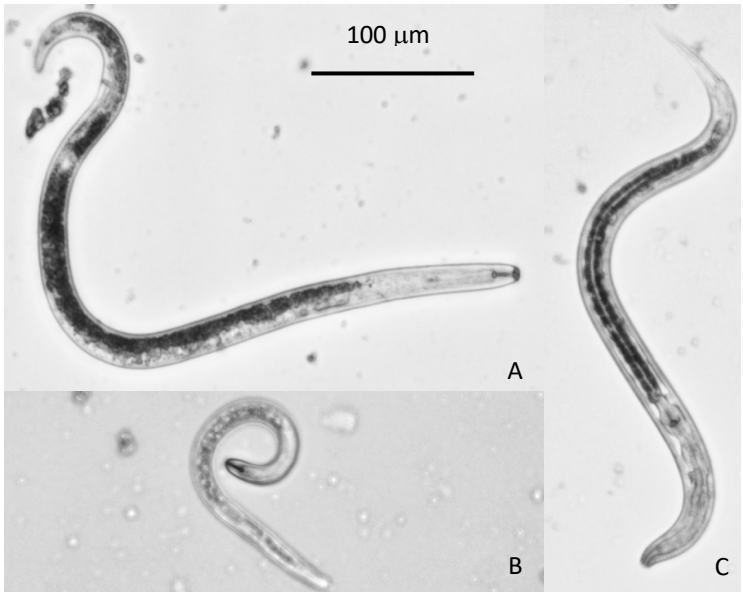


Figure F1: Nematode applications for the Summer Long Study, Phase 2. Average nematodes applied per plant was calculated by multiplying the mean density of five replicate 1 mL samples by the volume applied to each plant. Data presented are means ± standard error from five replicates. Photos A – C are at 10x magnification, documenting sample specimen extracted.

Post-harvest nematode densities

By the end of the experiment, nematode numbers in each pot had increased. Table F2 displays the average densities of nematodes in roots and soil, by treatment combination. While there were no significant differences in final densities by AMF treatment, there were significant differences in root densities due to inoculation with *P. rubi* (Table F3). Nematode soil density was not predicted by either AMF treatment nor Phytophthora treatment (Table F3).

Table F1: Density of nematodes recovered from root samples from harvested plants. Data displayed are means ± standard errors from ten replicates.

Treatment Combination		Nematodes / 100g soil	Nematodes / g root
Control	*P. penetrans*	129 ± 74	474 ± 161
	P. penetrans & *P. rubi*	67 ± 50	348 ± 116
Built	*P. penetrans*	224 ± 206	633 ± 116
	P. penetrans & *P. rubi*	89 ± 55	315 ± 40
Field	*P. penetrans*	145 ± 93	679 ± 157
	P. penetrans & *P. rubi*	181 ± 101	325 ± 39
Wild	*P. penetrans*	201 ± 267	321 ± 75
	P. penetrans & *P. rubi*	170 ± 161	366 ± 110

Table F3: Results of Wald Chi-square test, indicating significance of each treatment variable for predicting the response variable. Mixed models selected as described in the methods. Inclusion of the AMF:Phytophthora interaction did not improve the model for either response variable.

	AMF (df =3)		Phytophthora (df = 1)	
Response Variable	$\chi 2$	p	$\chi 2$	p
Root Density	2.95	0.399	**7.04**	**0.008**
Soil Density	4.20	0.241	2.29	0.130

Appendix G: Sensitivity Analysis

Results from G*Power indicated that there was ample power to detect main effects in our study, but the capacity of our design to detect interactions between pest and AMF treatments was limited medium-large effects (Cohen's f = 0.34). This suggests that in our analysis, where the interaction between AMF treatment and pest treatment was found to be non-significant, it would be safer to conclude that if any interaction existed, it was not a large effect.

All the interaction effect sizes were smaller than the minimum effect size that we could have detected with high probability given our research design (Table 5). For this reason, we used plots and simple main effects contrasts to assess potential interaction effects even where the interaction was not found to be significant. The main effects observed in response to AMF treatment were mostly large effects, and larger than the minimum effect size needed to have confidence that we could detect such an effect (Table 5).

Table G1: Results of sensitivity analysis. The smallest effect that we could have detected with high probability given our research design and measured error variance is reported as Cohen's f. Subsequent values are the effect size (partial Cohen's f) for each response variable. Values with a star are smaller effects than the minimum effect we could have detected with 80% confidence.

	Minimum Cohen's f for power = 0.80	Total Biomass (partial Cohen's f)	Root Biomass (partial Cohen's f)	Root:Shoot Ratio (partial Cohen's f)	SPAD (partial Cohen's f)
AMF (k = 4)	f = 0.27	0.42	0.44	0.15 *	0.43
Pest (k = 4)	f = 0.27	0.22 *	0.48	0.32	0.23 *
Interaction (k=16)	f = 0.34	0.16 *	0.24 *	0.25 *	0.32 *

Appendix H: Regression Results

After the best mixed model regressions were fit to each response variable, we used a Wald Chi-square test to describe the significance of each predictor variable in the model. AMF treatments had highly consistent effects, while there was never a significant main effect of *Phytophthora* on either plant growth or AMF colonization (Table H1).

Table H1: Results of Wald Chi-square test, indicating significance of each term in the best fit regression model fora given response variable. Models selected as described in the methods; inclusion of AMF:Nem:Phy did not improve the models.

Response Variable	AMF (df =3) $\chi2$	p	Phytophthora (df = 1) $\chi2$	p	Nematode (df =1) $\chi2$	p	Nem:Phy Interaction (df = 1) $\chi2$	p
Physical Measures								
Height	**14.69**	**0.002**	0.27	0.603	0.15	0.701	1.49	0.222
Nodes / 10cm	2.22	0.528	0.01	0.928	0.19	0.660	1.72	0.190
SPAD	**24.71**	**<0.001**	0.50	0.481	1.61	0.205	**4.85**	**0.028**
Dry Weights								
Root Biomass	**33.66**	**<0.001**	1.33	0.248	**19.65**	**<0.001**	3.34	0.068
Shoot Biomass	**32.63**	**<0.001**	0.06	0.800	0.11	0.746	**4.76**	**0.029**
Total Biomass	**47.90**	**<0.001**	0.05	0.826	7.5	0.006	**6.3**	**0.012**
Shoot Nutrients								
Nitrogen (%)	**9.92**	**0.019**	0.24	0.624	0.02	0.885	1.58	0.209
Phosphorous (%)	**84.06**	**<0.001**	0.42	0.516	1.49	0.222	0.72	0.396
Potassium (%)	**8.00**	**0.046**	3.45	0.063	**8.14**	**0.004**	2.75	0.097
Calcium (%)	**10.13**	**0.017**	1.12	0.289	**4.67**	**0.031**	3.76	0.052
Magnesium (%)	**11.86**	**0.008**	0.04	0.841	2.37	0.124	**5.91**	**0.015**
Colonization								
Total Colonization (%)	**84.48**	**<0.001**	0,03	0.855	2.82	0.093	0.08	0.773
Arbuscules (%)	**34.15**	**<0.001**	0.68	0.410	0.17	0.681	2.54	0.111
Vesicles (%)	**76.50**	**<0.001**	2.25	0.133	6.72	0.009	2.52	0.112

Appendix I: Spore Production

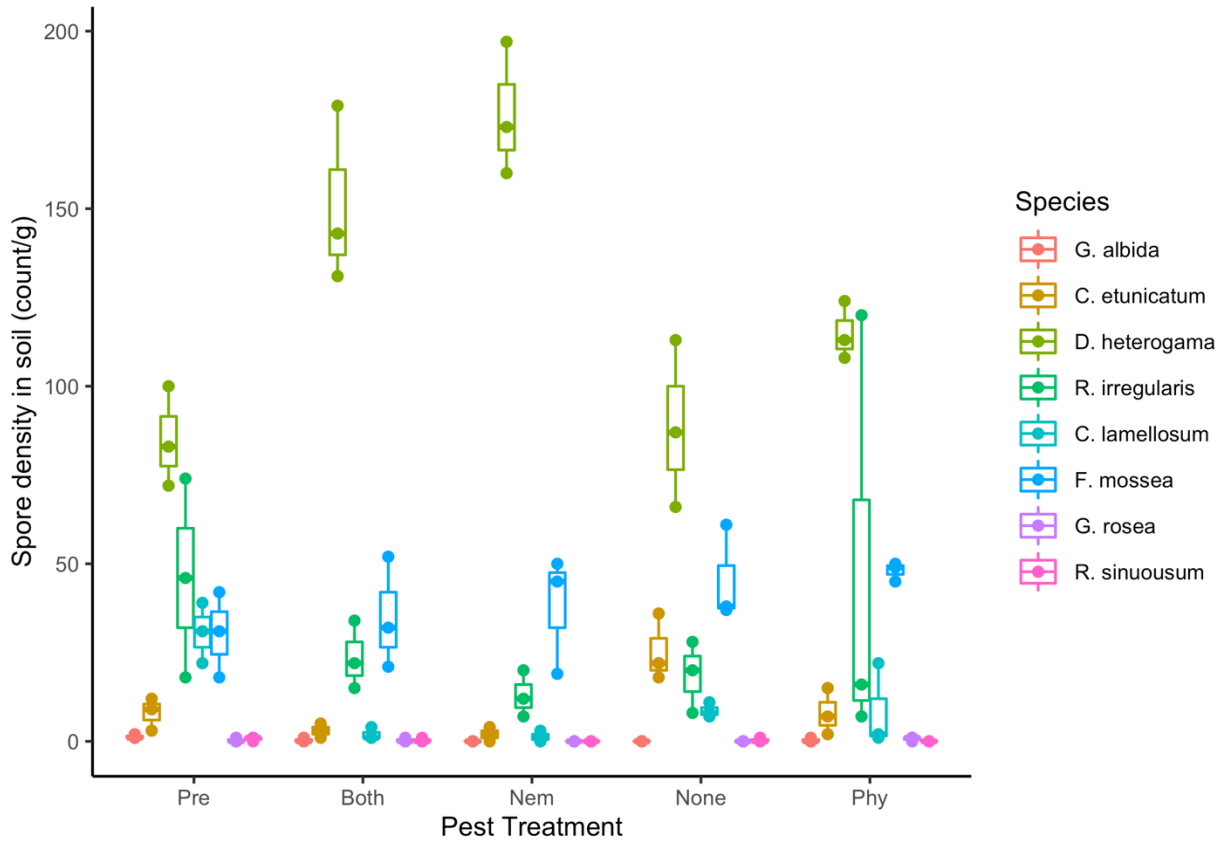
Soils from every treatment combination in the Summer Long Study were dried and spore abundances were later assessed. Plants with Built AMF had the highest final spore densities, regardless of stress treatment (Table I1).

Table I1: AMF spore densities in soil following the Summer Long Study, by treatment combination. Plants were first grown with the AMF treatment in the first column, then introduced to the stress treatment in the second column at week 10. Soils were pooled by treatment combination. Data displayed are means ± standard errors from 3 replicates.

Treatment combination		Spores per g dry soil
Control	Control	141 ± 6
	Pratylenchus penetrans	57 ± 2
	Phytophthora rubi	46 ± 7
	Both	57 ± 3
Built	Control	187 ± 3
	Pratylenchus penetrans	231 ± 4
	Phytophthora rubi	228 ± 43
	Both	215 ± 15
Farm	Control	88 ± 9
	Pratylenchus penetrans	94 ± 9
	Phytophthora rubi	91 ± 16
	Both	110 ± 13
Wild	Control	34 ± 8
	Pratylenchus penetrans	38 ± 6
	Phytophthora rubi	35 ± 6
	Both	41 ± 22

For the Built treatment, spores were identified to species, because we knew the species profile of the original inoculum which was comprised of species with morphologically distinct spores. Community structure, approximated as the relative abundance of spores, changed following 24 weeks of culture with *R. idaeus* subjected to various pest/pathogens. The most obvious change was that plants receiving nematodes, with or without *P. rubi*, had a significantly higher density of *D. heterogama* spores at the end of the experiment (Figure I1). Shannon's Diversity Index H was calculated using the relative abundance of each species spores. Community diversity, as described by Shannon's H, was reduced by the presence of pests, especially nematodes (Figure I1).

Because all spores in the Built inoculum were identified by matching their morphology to the 8 species in the inoculum, there is a chance that some windborne contaminants were mistakenly included in those numbers. That said, just as the contaminants did not establish and sporulate as prolifically in Control treatment pots where there were pests also competing for resources (Figure 7), those contaminants would have faced similar competition from the established AMF communities in the Built, Farm and Wild treatments. Windborne contaminants identified in the Control treatments were mostly small (<100 microns) and medium (100-150 microns), yet in the Built treatment the species with the largest increase in spore density was *D. heterogama*, which has amber colored, medium-large spores averaging 159 microns in diameter (West Virginia University INVAM). That said, we should interpret these preliminary community results with caution, due to the risk of including mis-identified contaminants.



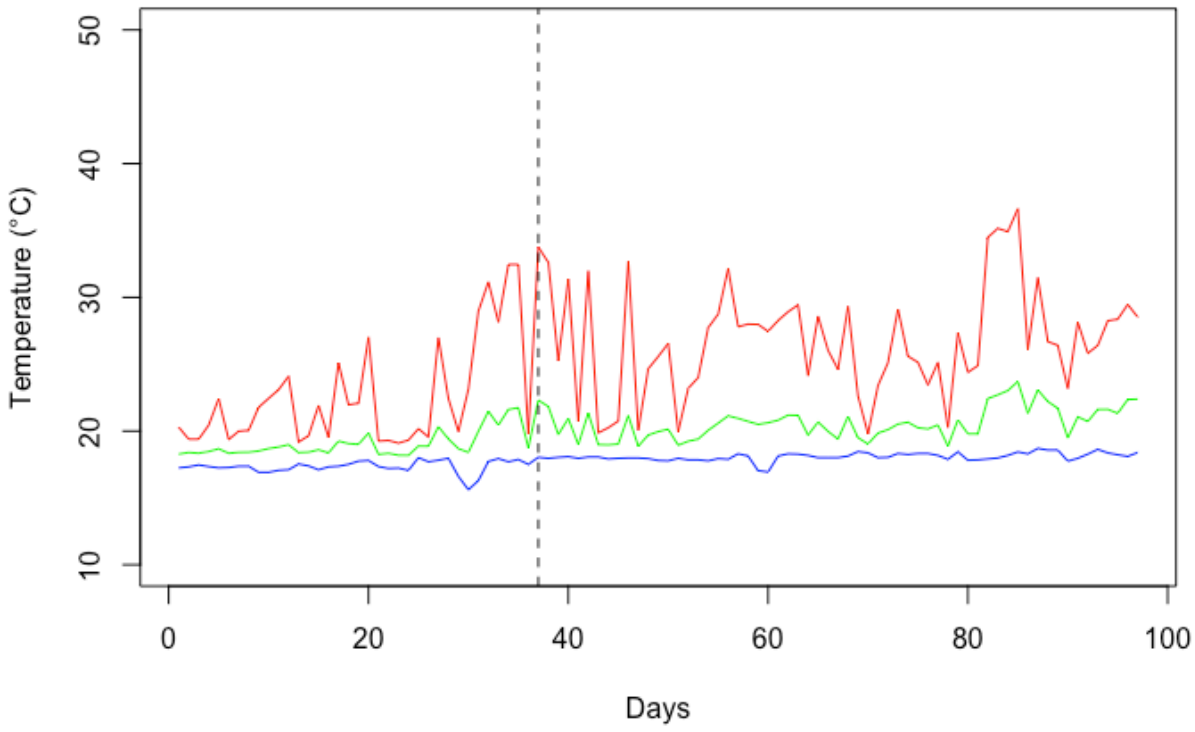
Treatment	Pre	Both	Nem	None	Phy
Shannon's H	1.46	0.92	0.73	1.35	1.26

Figure I1: Summary of spore abundance within plants receiving Built AMF in Phase 1. Phase 2 stress treatments are on the x-axis, in addition to "Pre" which is the species abundance measured within an equivalent volume of the Built inoculum before the start of the experiment. Boxplots are based upon median and interquartile range of three replicates per treatment combination. Table contains Shannon Diversity index for "Pre" and each treatment.

Appendix J: Spring Short Study

Environmental Conditions

The daily average temperatures were within the “ideal growing range” of raspberry on 95% of days (Figure A1), while never topping 38°C (100°F).



Month	Average Temp (°C)	Average Daily High (°C)	Average Daily Low (°C)
February	18.6	21.1	17.3
March	20.0	25.6	17.7
April	20.7	27.3	18.1
May	21.8	27.4	18.3

Figure J1: Greenhouse temperature trends for the Spring Short Study (2020). The plot shows trends in daily average (green), highs (red) and low (blue) temperatures over the course of the experiment, with days on the x-axis. Vertical dashed line marks the date at which plants were introduced to their Phase 2 stress treatments. The table further summarizes these trends by month (means).