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The effects of ericoid inoculum source and nutrient addition on growth and reproduction in highbush blueberry

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THE EFFECTS OF ERICOID INOCULUM SOURCE AND NUTRIENT ADDITION ON GROWTH AND REPRODUCTION IN HIGHBUSH BLUEBERRY

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Abstract

Ericoid mycorrhizal fungi (EMF) form symbiotic relationships with ericaceous plants such as *Vaccinium corymbosum*, or highbush blueberry and assist in nutrient acquisition. EMF help plants thrive in stressful environments by increasing the area in which roots can uptake water and nutrients. In plant-mycorrhizal symbioses, nutrient uptake may depend on the identity of the fungal partner. Therefore, differently sourced mycorrhizal fungi could show differences in nutrient uptake ability. Here, I hypothesized that inoculation of *V. corymbosum* with EMF would enhance plant growth and investment in reproduction, and that effect would be more pronounced for plants in low nutrient conditions. I also hypothesized that inoculum source would affect plant growth and reproduction under varying nutrient conditions. To test this, I used 135 potted highbush blueberry plants that were inoculated at planting with either 1) commercial inoculum, 2) local inoculum, or 3) uninoculated control. Within these inoculum treatments and the control, I haphazardly assigned 15 plants to one of three fertilizer treatments using SUPERthrive fertilizer (N:P:K ratio of 4:1:1) at: i) the amount recommended for field grown plants, ii) half the recommended amount of fertilizer, and iii) no fertilizer. I predicted that plants inoculated with EMF from local soils would be better able to access nutrients than those inoculated with commercial inoculum or non-inoculated controls, and the effects would be measurable through increased plant growth and reproduction.

Inoculation enhanced plant size ($F_{2,161}$=3.157; P=0.045), the number of flowers produced ($F_{2,112}$=3.736; P=0.027), and the number of berries produced ($F_{2,113}$=3.653; P=0.029). However, fertilization had no significant effects on any of the response variables measured. Plants that received the local soil inoculum were significantly larger than commercially inoculated plants ($F_{2,161}$=3.157; P=0.045). Plants in the local inoculum treatment produced significantly more flowers ($F_{2,112}$=3.736; P=0.027) and berries ($F_{2,113}$=3.653; P=0.029) than plants in the commercial inoculum treatment, and plants responded differently to the inoculum treatments in the two years of the study ($F_{2,121}$=6.371; P=0.002). Additionally, initial plant size had a significant effect on the total number of berries produced ($F_{1,106}$=7.047; P=0.009). The relationship between plant size and number of berries differed between inoculum treatments. Plants in the commercial inoculum and control treatment showed a positive correlation between plant size and berries produced, while the number of berries on plants in the local inoculum treatment remained constant as plant size increased ($F_{2,107}$=3.320; P=0.040). Despite the order of magnitude differences in fertilizer applied, concentrations of N, P, K measured in soils taken from plants in the different treatments were all within the optimal range recommended for highbush blueberry. However, NH_4 and K soil concentrations differed between inoculum treatments, revealing that differently sourced mycorrhizal fungi may differ in nutrient uptake.

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Chapter 1: Thesis Introduction

Mycorrhizal associations with plants are extremely widespread throughout the world. More than 90% of all land plant species form interactions with mycorrhizal fungi (Brundrett 2009), leading to a bidirectional exchange of nutrients between fungi and plants (Wang and Qiu 2006). Fungi interacting with plant roots in the soil transfer important nutrients (e.g., nitrogen, phosphorus) to the host plant and the plant transfers photosynthetic products such as fixed carbon to the fungus (Smith and Read 2008). The partnership with mycorrhizal fungi can increase the area in which roots can uptake water and nutrients, providing a benefit to host plants especially in stressful environmental conditions such as drought (Auge 2001; Al-Karaki et al. 2004), heavy metal toxicity (Cairney and Meharg 2003), salt stress (Giri et al. 2007), and soil nutrient deficiency (Wright et al 1998).

Most of the literature on mycorrhizal fungi focuses on arbuscular mycorrhizal fungi (AMF), which interact with the largest number of plants compared to other less common fungi such as ericoid or orchid mycorrhizal fungi (van der Heijden et al. 2015). The symbiosis between AMF and plants is thought to have evolved about 450 million years ago and is credited with assisting in the transfer of plants from water to land (Redecker et al. 2000). AMF have not been found to be host-specific, however host preferences and specificity have been reported (Helgason et al. 1998; Torrecillas et al. 2012). There are between 300 - 1600 different fungal taxa within AMF (Kivlin et al. 2011; Öpik et al. 2013;), and an estimated 200,000 host plant species (Brundrett 2009), suggesting a low host specificity. Even in the absence of a growth response, AMF have

been found to reduce nutrient losses to the plant by lessening harmful effects of leeching (van der Heijden 2010).

Unlike AMF, ericoid mycorrhizal fungi have been minimally studied (Vohnıḱ 2020). Ericoid mycorrhizal fungi interact with species in the Ericaceae family, such as *Calluna, Rhododendron,* and *Vaccinium*. Interactions with ericoid fungi are most common in acidic and infertile heathland environments. Many of the known ericoid fungi belong to the Helotiales (Ascomycetes) and are considered saprotrophs (van der Heijden et al. 2015). Because of the lack of data currently available it is difficult to estimate the total existing number of ericoid mycorrhizal fungi, however greater than 150 fungal taxa have been identified as associating with ericaceous plants (Walker et al. 2011) and around 3,900 plant species are known to host ericoid mycorrhiza (Brundrett 2009). Because ericoid mycorrhizal fungi form associations with plants in the Ericaceae family, only about 1% of plants on earth form ericoid mycorrhizal associations, compared to the 74% of all plant species that form arbuscular mycorrhizal associations (Smith and Read 2008; Brundrett 2009).

Different types of mycorrhizal fungi can provide various benefits to plants by enhancing nutrient acquisition, leading to increased plant productivity and reproduction. Mycorrhizal fungi are important drivers of carbon and nutrient cycles and play a large role in ecosystem processing. Much of the N and P up taken by plants is facilitated by mycorrhizal fungi, especially in conditions of low nutrient availability. Ericoid fungi can obtain large amounts of essential nutrients, sometimes providing up to 80% of plant N and P (Simard et al. 2002).

Though there are many instances of plant-fungal symbioses having a mutualistic outcome, this is not always the case. Some studies provide evidence of plant-fungal mutualisms becoming parasitic under certain environmental conditions (Johnson 1993; Antunes et al. 2012). Parasitic outcomes may be related to the trade balance of N, P, and C between plant and fungal symbiont (Johnson 2010; Kiers et al. 2011). When N and P are supplied to the plant in sufficient amounts, C is easily produced and transferred to the fungal partner. With adequate nutrient levels, the symbiosis becomes unnecessary to the host plant, and this results in continuous benefits for the fungal partner with no benefit of fungal mediated nutrient transfer to the plant. These conditions of sufficient macronutrient levels could lead to parasitism (Antunes et al. 2012). Studies show that less mutualistic mycorrhizal fungi can be selected for in conditions where they are not a necessity to plant nutrient uptake (Johnson 1993; Treseder and Allen 2002). Additionally, Antunes et al. (2012) provide evidence that ongoing severe nutrient deficiencies can alter the association between plants and AMF communities, allowing the fungal partner to cheat the host plant by accessing C without providing nutrients in return. However, the maintenance of sufficient nutrient levels using chemical fertilizer may result in AMF becoming parasitic on their hosts (Johnson 1993).

An important element of plant-fungal symbioses is that there are more than just two players in the ecological interaction occurring in the plant rhizosphere. Different groups of soil bacteria have been known to interact with AMF, including plant-growth-promoting rhizobacteria (PGPR), mycorrhizal helper bacteria, and deleterious bacteria,

leading to recent studies exploring the tripartite symbiosis between plants, mycorrhizal fungi, and bacteria (Miransari 2010; Scheublin et al. 2010). PGPR can positively affect AMF root colonization and enhance plant N and P uptake (Miransari 2010). The synergistic effects of AMF and rhizobacteria can also inhibit fungal plant pathogens and soilborne diseases (Budi et al. 1999). Additionally, mycorrhizal fungi can interact with other types of fungi in the mycorrhizosphere, which could positively affect overall plant growth and nutrition (Bao et al. 2022).

The relative effects of "foreign vs local" species interactions have been studied in numerous systems, from local adaptation of invasive plant species (Oduor et al. 2016) to preferential bird songs (Parra et al. 2017). Oduor et al. (2016) found that invasive plant species are locally adapted just as frequently as native plants across 134 plant species in 52 families. Parra et al. (2017) found that songbirds do not respond differently to local vs foreign songs. The "foreign vs local" concept has also been studied in mycorrhizal fungi, examining plant response to native vs exotic mycorrhizal fungi using various host plants. Locally sourced mycorrhizal fungi often out-perform a commercial inoculum and lead to increased plant growth (Klironomos 2003; Taheri and Bever 2011; Middleton et al. 2015; Emam 2016).

In this study, I aimed to examine the effects of foreign vs local ericoid mycorrhizal fungi on plant growth and reproductive traits in *V. corymbosum.* In addition to the effects of differently sourced mycorrhizal inoculums, I also tested plant response to varying levels of fertilizer added. Because plant-fungal mutualisms can shift with varying

amounts of nutrients available in the soil, I examined whether the effects of limited fertilizer differed between inoculum treatments. My analysis showed that there were no significant differences in nutrient concentrations between the fertilizer treatments, and soil tests suggested that nutrient levels across all plants were sufficient. I did find that in some cases the two inoculum sources differed in plant response. Overall, plants that received the local soil inoculum were larger and produced more flowers and berries than plants that received the commercial inoculum. Additionally, in locally inoculated plants, plant reproductive traits remained stable in response to changes in plant size.

The results of the present study add to the growing knowledge about how belowground species interactions influence plant growth and reproduction as well as how ericoid mycorrhizal fungi aids in plant processes. The next step is to determine, through gene sequencing, which fungal taxa are present in the soil to understand the players involved in this system and relate plant functional traits to fungal identity within the rhizosphere community.

1.1 Works Cited

Al-Karaki, G., McMichael, B., and Zak, J. 2004. Field response of wheat to arbuscular mycorrhizal fungi and drought stress. Mycorrhiza, 14: 263-269.

Antunes, P.M., Lehmann, A., Hart, M.M., Baumecker, M. and Rillig, M.C. 2012. Long-term effects of soil nutrient deficiency on arbuscular mycorrhizal communities. Functional Ecology, 26: 532-540.

Auge, R.M. 2001. Water relations, drought, and vesicular-arbuscular mycorrhizal symbiosis. Mycorrhiza, 11: 3-42.

Bao, Xiaozhe, Jixiang Zou, Bin Zhang, Longmei Wu, Taotao Yang, and Qing Huang. 2022. Arbuscular Mycorrhizal Fungi and Microbes Interaction in Rice Mycorrhizosphere. Agronomy, 12: 1277.

Brundrett, M.C. 2009. Mycorrhizal associations and other means of nutrition of vascular plants: understanding the global diversity of host plants by resolving conflicting information and developing reliable means of diagnosis. Plant Soil, 320: 37–77.

Budi, S.W., Van Tuinen, D., Martinotti, G., Gianinazzi, S. 1999. Isolation from the Sorghum bicolor mycorrhizosphere of a bacterium compatible with arbuscular mycorrhiza development and antagonistic towards soilborne fungal pathogens. Applied and Environmental Microbiology, 65: 5148–5150.

Cairney, J. W. G., and Meharg, AA. 2003. Ericoid mycorrhiza: A partnership that exploits harsh edaphic conditions. European Journal of Soil Science. 54: 735-740.

Douglas, A.E. 2010. The Symbiotic Habit. Princeton University Press, Princeton, NJ. Mutualisms.

Emam, T. 2016. Local soil, but not commercial AMF inoculum, increases native and non-native grass growth at a mine restoration site. Restoration Ecology, 24: 35-44.

Giri, B., Kapoor, R., and Mukerji, KG. 2007. Improved tolerance of *Acacia nilotica* to salt stress by arbuscular mycorrhiza, *Glomus fasciculatum* may be partly related to elevated K/Na ratios in root and shoot tissues. Microbial Ecology, 54: 753-760.

Helgason T, Daniell TJ, Husband R, Fitter AH, Young JPW. 1998. Ploughing up the wood-wide web? Nature, 394: 431.

Johnson, N.C. 1993. Can fertilization of soil select less mutualistic mycorrhizae? Ecological Applications, 3: 749-757.

Johnson, N.C. 2010. Resource stoichiometry elucidates the structure and function of

arbuscular mycorrhizas across scales. New Phytologist, 185: 631–647.

Kiers ET, Duhamel M, Beesetty Y, Mensah JA, Franken O, Verbruggen E, Fellbaum CR, Kowalchuk GA, Hart MM, Bago A et al. 2011. Reciprocal rewards stabilize cooperation in the mycorrhizal symbiosis. Science, 333: 880–882.

Kivlin SN, Hawkes CV, Treseder KK. 2011. Global diversity and distribution of arbuscular mycorrhizal fungi. Soil Biology & Biochemistry, 43: 2294–2303.

Klironomos J.N. 2003. Variation in plant response to native and exotic arbuscular mycorrhizal fungi. Ecology, 84: 2292-2301.

Middleton, E. L., S. Richardson, L. Koziol, C. E. Palmer, Z. Yermakov, J. A. Henning, P. A. Schultz, and J. D. Bever. 2015. Locally adapted arbuscular mycorrhizal fungi improve vigor and resistance to herbivory of native prairie plant species. Ecosphere, 6: 276.

Miransari, M. Interactions between arbuscular mycorrhizal fungi and soil bacteria. 2011. Applied Microbiology and Biotechnology, 89: 917–930.

Oduor, A.M.O. R. Leimu, and M. van Kleunen. 2016. Invasive plant species are locally adapted just as frequently and at least as strongly as native plant species. Journal of Ecology, 104: 957-968.

Öpik M, Zobel M, Cantero JJ, Davison J, Facelli JM, Hiiesalu I, Jairus T, Kalwij JM, Koorem K, Leal ME et al. 2013. Global sampling of plant roots expands the described molecular diversity of arbuscular mycorrhizal fungi. Mycorrhiza, 23: 411–430.

Parra, M., A.C. Dalisio, W.E. Jensen, and T.H. Parker. 2017. Male terrestrial aggression does not drive conformity to local vocal culture in a passerine bird. Ethology, 123: 800-810.

Redecker, D., R. Kodner, and L.E. Graham. 2000. Glomalean fungi from the Ordovician. Science, 289: 1920-1921.

Simard SW, Jones MD, Durall DM. 2002. Carbon and nutrient fluxes within and between mycorrhizal plants. Mycorrhizal Ecology, 33–74.

Scheublin, T.R., Sanders, I.R.,Keel, C., and van der Meer, J.R. 2010. Characterisation of microbial communities colonising the hyphal surfaces of arbuscular mycorrhizal fungi. ISME Journal, 4: 752–763.

Smith, S. E., and D. J. Read. 2008. Mycorrhizal symbiosis, 3rd ed. Academic Press, San Diego, CA, USA.

Taheri, W.I., and Bever, J.D. 2011. Adaptation of *Liquidambar styraciflua* to coal tailings

is mediated by arbuscular mycorrhizal fungi. Applied Soil Ecology, 48: 251-255.

Torrecillas E, Alguacil MM, Roldan A. 2012. Host preferences of arbuscular mycorrhizal fungi colonizing annual herbaceous plant species in semiarid Mediterranean prairies. Applied and Environmental Microbiology, 78: 6180–6186.

Treseder, K.K. & Allen, M.F. 2002. Direct nitrogen and phosphorus limitation of arbuscular mycorrhizal fungi: a model and field test. New Phytologist, 155: 507–515.

van der Heijden MGA. 2010. Mycorrhizal fungi reduce nutrient loss from model grassland ecosystems. Ecology, 91: 1163–1171.

van der Heijden, M.G.A., Martin, F.M., Selosse, M.-A. and Sanders, I.R. 2015. Mycorrhizal ecology and evolution: the past, the present, and the future. New Phytologist, 205: 1406-1423.

Vohnıḱ, M. 2020. Ericoid mycorrhizal symbiosis: theoretical background and methods for its comprehensive investigation. Mycorrhiza, 30: 671–695.

Walker JF, Aldrich-Wolfe L, Riffel A, Barbare H, Simpson NB, Trowbridge J, Jumpponen A. 2011. Diverse Helotiales associated with the roots of three species of Arctic Ericaceae provide no evidence for host specificity. New Phytologist, 191: 515–527.

Wang B and Qiu YL. 2006. Phylogenetic Distribution and Evolution of Mycorrhizas in Land Plants. Mycorrhiza, 16: 299-363.

Wright DP, Read DJ, Scholes JD. 1998. Mycorrhizal Sink Strength Influences Whole Plant Carbon Balance of *Trifolium repens L*. Plant, Cell, and Environment, 21: 881-891.

Chapter 2: Effects of ericoid inoculum source and nutrient addition on growth and reproduction in highbush blueberry

2.1 Introduction

The association of plants with mycorrhizal fungi is one of the oldest and most important symbioses on earth. More than 90% of all land plant species form interactions with mycorrhizal fungi (Brundrett 2009). The relationship between mycorrhizal fungi and their host plant is based on a bidirectional exchange of resources; the mycorrhizal fungus provides the host plant with nutrients such as phosphorus and nitrogen and in return the plant transfers photosynthetically fixed carbon to the fungus (Smith and Read 2008).

Mycorrhizal fungi assist plants in the acquisition of several nutrients including phosphorus, nitrogen, potassium, calcium, sulfate, iron, copper, and zinc (Marschner and Dell. 1994). Mycorrhizal hyphae absorb, transport, and utilize nitrogen in the form of NH4+ (Ames et al. 1983). Under field conditions, mycorrhizal plants were shown to have increased nitrogen uptake compared to non-mycorrhizal plants (Barea et al. 1987). Mycorrhizal fungi are credited with a major role in the extraction of nitrogen from organic matter from soils by both producing and regulating different ectoenzymes such as proteinases that break down proteins (Leake and Read 1990). Ericoid mycorrhizal fungi produce ectoenzymes into the surrounding soil medium which allow host plants to access forms of organic nitrogen and phosphorus that are usually unavailable to non-mycorrhizal plant roots (Marschner and Dell 1994).

In addition to providing plants with nutrients, mycorrhizae can lessen the negative effects of environmental conditions such as drought (Al-Karaki et al. 2004), soil salinity

(Giri et al. 2007), and toxic metal pollution (Cairney and Meharg 2003). These conditions can lead to low soil nutrient concentrations, and mycorrhizal fungi can reduce resource limitation of plants by increasing nutrient uptake from the soil (Wright et al. 1998). While mycorrhizal inoculation has been shown to increase nutrient uptake in some cases (George et al. 1995; Jansa and Vosatka 2000; Cavagnaro et al. 2001), these results are not consistent throughout the literature (Bell and Pate 1996; Hawkins et al. 2000; Reynolds et al. 2005; Smith and Smith 2011; Hodge and Storer 2015).

For example, under conditions of high salinity and low nutrients inoculation with mycorrhizal fungi ameliorated harmful effects of salinity by enhancing plant access to phosphorus (Giri et al. 2007) and improving soil microbial activity (Al-Maliki and Al-Masoudi 2018), however in an extreme salt stressed environment mycorrhizal colonization significantly decreased (Krishnamoorthy et al. 2014). Mycorrhizal inoculation was also able to reduce the negative effects of drought stress by enhancing plant growth, nutrient uptake, and soil water retention in winter wheat (Al-Karaki et al. 2004) and *Citrus trifoliata* (Wu et al. 2008) but did not mitigate the effects of drought on growth in potted *Trifolium* sp. (Jongen et al. 2022). In addition, mycorrhizal inoculation enhanced plant growth in lead-contaminated soils by increasing nutrient uptake and mitigating lead toxicity through sequestering lead in plant roots (Chen et al. 2005).

Conversely, previous studies have found that excess nutrient input can have a negative effect on ericoid mycorrhizal colonization in soils (Zinati et al. 2011; Van Geel et al. 2020). Van Geel et al. (2020) found that there was a substantial loss of ericoid

fungal taxa as nitrogen and phosphorus increased in soil prevalence, suggesting that mycorrhizal fungi are too costly when soils are nutrient rich. Nutrients in excess could negatively impact the growth and health of plant species and cause ericoid fungi to switch from a mutualistic relationship to commensalism or even parasitism (Wei et al. 2013; Johnson et al. 2015). If the plant is getting consistent nutrients without help from the fungi, the symbiosis could become parasitic where the fungus keeps receiving excess nutrients and the plant no longer benefits (Antunes et al. 2012).

Specific to the present study, ericoid mycorrhizal fungi form symbiotic relationships with members of the Ericaceae family, and differ from ectomycorrhizal, arbuscular, and orchid mycorrhizal fungi in plant association and ecosystem processes such as nutrient cycling, plant productivity, and reproduction (Smith and Read, 2008; Grelet et al., 2009; van der Heijden et al., 2015). Ericaceous plants tend to inhabit acidic soils containing recalcitrant nutrients; the association with ericoid mycorrhizal fungi enhances plant nutrient acquisition in these stressful environments (Marschner and Dell, 1994; Read 1996; Cairney and Meharg 2003; Zhang et al. 2009) partly because ericoid fungi thrive in the same level of soil acidity as their plant partners (van der Heijden 2015).

Differently sourced mycorrhizal inoculums could have varying efficiency and benefits to the plant. Locally sourced mycorrhizae better benefit plant growth and protection than a commercial inoculum or a control (Middleton et al. 2015). Plants experienced enhanced growth, mycorrhizal colonization rates, and increased biomass

when grown in their native soil than when grown in soil from a foreign site (Klironomos 2003; Taheri and Bever 2011; Emam 2016). However, Frew (2020) concluded that a commercially sold foreign inoculum resulted in increased plant growth and resource allocation.

Ericoid mycorrhizal inoculation enhanced flower production of some cultivars of *Vaccinium corymbosum* in an experiment preceding the present study (Brody et al. 2019), suggesting that inoculated plants had greater access to resources. To test if inoculation by ericoid mycorrhizal fungi enhances growth and reproductive traits in *V. corymbosum*, I designed an experiment in which the amount of fertilizer application varied to test the role of mycorrhizal fungi in nutrient acquisition by the plant. I asked: Does the level of nutrient addition influence the effect of the inoculum? And does the response depend on the source of the inoculum?

I hypothesized that ericoid mycorrhizal fungi inoculation would enhance *V. corymbosum* plants' ability to grow and reproduce under different nutrient conditions. Additionally, the source of the inoculum would affect the growth and reproductive traits of the plant. Specifically, I measured the effects of limited to no fertilizer on plant growth and reproductive traits of *V. corymbosum*. I expected that the plants inoculated with ericoid mycorrhizal fungi would show enhanced growth in low-nutrient conditions compared to non-inoculated control plants, and the plants that received a local inoculum would show enhanced growth and reproduction compared to commercially inoculated plants. Because of varying benefits of the fungal symbionts under different levels of

fertilizer, I predicted that nutrients in the soil, specifically nitrogen and phosphorus, would vary in concentration between treatments and over time.

Materials and Methods

2.2.1 Experimental Design

To examine the individual and interactive effects of inoculation with ericoid mycorrhizal fungi and fertilization, I conducted a common garden field experiment at the University of Vermont's Horticulture Research Farm in South Burlington, Vermont, USA. In March 2018, 380 4-year-old *Vaccinium corymbosum* plants (var. Blue Crop) were obtained from Hartmann's Plant Company in Michigan and randomly assigned to one of five different mycorrhizal inoculation treatments: 1) inoculated with a commercial ericoid inoculum, 2) inoculated with a soil taken from the rhizosphere of Blue Crop bushes growing at the Waterman Berry Farm in Johnson, VT, 3) a combination of treatments 1 and 2, 4) a control treatment with no inoculum, and 5) a peat control which is the base of the commercial inoculum. Ninety plants were randomly assigned to each treatment with the exception of the peat control treatment which received 20 plants. Plants were removed from pots, soil was washed from roots, and the root ball was covered with 6 ounces of either inoculum, soil, or peat, applied to the wet roots by hand, and then placed in a 7-gallon pot. The pot was then filled with a potting mix that was 12:6:3:1 peat:compost:perlite:vermiculite. Compost was purchased commercially and contained leaf and yard waste as well as food scraps, wood chips, horse manure, and high carbon wood ash. Plants were arranged in 10x9 treatment blocks, except the peat control, which was arranged in a 10x2 array. Plants were grown at the University of Vermont

Horticultural Farm in South Burlington, VT for the remainder of the summer, and then pots were placed into individual holes in the ground and covered with straw mulch. Each year after, plants were fertilized before fruiting with 10 mL of fertilizer per pot with SUPERthrive fertilizer (N:P:K ratio of 4:1:1) at the concentration recommended for field grown plants (Scagel 2005).

Starting in May of 2020, to examine if ericoid mycorrhizal fungi can compensate for low nutrient conditions, and whether the source of ericoid mycorrhizae matters, I examined plant growth and reproduction in three inoculum treatments and three fertilizer treatments. Out of the 90 plants in each mycorrhizal treatment group, I haphazardly chose 45 plants from the commercial inoculum, local soil inoculum, and control treatments, for a total of 135 plants. Of these, I haphazardly assigned 15 plants per mycorrhizal treatment to one of three treatments: i) no fertilizer, ii) half the amount of recommended fertilizer, and iii) full amount of recommended fertilizer. Fertilizer was applied prior to fruiting on May 25, 2020, and again on May 29, 2021.

2.2.2 Growth Measurements

To examine if inoculation, fertilization, or the combination thereof affected plant size, I counted the number of stems and measured the height of all stems that were initiated from the rootstock which I refer to as “main stems” (as opposed to lateral branches which arise from the main stems). Main stems were counted and measured in the beginning of the season in both years (on 26 May 2020 and 04 June 2021) and at the end of each growing season (26-28 August 2020 and 31 August – 09 September 2021). Plant size was calculated by summing the heights of all main stems on the plant.

2.2.3 Flower, Berry, and Abort Counts

Vaccinium corymbosum preforms flower buds in the fall (Kovaleski et al., 2015). To examine the effects of the inoculum and fertilization on reproduction, all flower buds and flowers were counted on 8 June 2020 and between 19-25 May 2021. Flowering occurred much later in 2020 than in 2021. Buds that were still closed at this time were included in the floral count.

At the end of the flowering season, the total number of berries and the number of aborted berries on each plant was summed to calculate total number of flowers produced (number of fruits + number of aborts) and fruit set (number of fruits/total number of flowers). Aborts were classified as either expanded or unexpanded. Expanded aborts began to develop into a berry but shriveled and died before ripe. Unexpanded aborts were flowers that shriveled before a berry began to form. Aborts were counted from flowering until the end of berry counting, from 12 June - 07 July 2020 and 01 June – 16 July 2021 and were removed from the plant to prevent double counting.

Berries were counted from 03 July – 07 July 2020, when the majority of the berries were ripe and aborted berries had mostly all been counted and removed. In the next summer, berries were counted on 05 July and 07 July 2021.

2.2.4 Berry Collection and Processing

To examine the effects of inoculation and fertilization on berry mass, sugar content, and number of fertilized seeds, berries were collected from 20 July - 7 August 2020 and 12 July – 5 August 2021 every two days until sixteen fully ripe berries (or all

berries if the plant produced less than sixteen in total) were collected from every plant. To avoid bias, berries were haphazardly hand-picked off the plant, semi "blindly" (by turning away, reaching for a berry, and repeating this across the branches containing berries). Berries were then kept frozen until processing. Each berry was weighed, and its sugar content was quantified using a hand-held refractometer. Seeds were counted and categorized as either fertilized and mature or unfertilized.

2.2.5 Soil Collection and Analysis

To examine if fertilization levels affected soil nutrients among inoculation treatments, I took soil samples seven weeks after fertilizing in 2020 and 2021. On 13 July 2020 I took a soil sample from three plants per fertilizer-inoculation treatment, for a total of 27 plants. The next summer, on July 17, 2021, I sampled five plants per fertilizer-inoculation treatment, for a total of 45 plants. Soil samples were taken using a soil corer at four different locations around the pot and then homogenized. Soil analysis was performed at The University of Maine using a routine field soil test. Samples were air dried to constant weight and sieved through 2 mm. Media pH was measured in distilled water, and nitrate and ammonium nitrogen were extracted in potassium chloride and determined colorimetrically by Flow Injection Analysis. All other nutrients were extracted in pH 4.8 ammonium acetate (modified Morgan extract) (From Maine Soil Testing Service).

2.2.6 Statistical Analyses

All statistical analyses were performed using JMP Pro 15 (JMP <*15*>, 1989-

2022). Response variables included plant size, flower and berry production, fruit set, berry mass, number of fertilized seeds per berry, sugar content, and the concentration of nutrients N, P, K (ppm) in the soil. For each response variable, inoculation treatment, fertilizer treatment, year, and all two-way interactions were analyzed as main effects and plant size at the beginning of the 2020 growing season was included as a covariate. Plant number was included as a random effect, as the same plants were repeatedly analyzed for two years. Significant main effects were further analyzed using Least-Squares Means Tukey HSD Tests to determine the differences among means.

2.3 Results

2.3.1 Plant Size

The source of inoculum significantly affected plant size ($F_{2,161}$=3.157; P=0.045; Figure 2.1). Plants in the local inoculum treatment were significantly larger (mean = 215.041 ± 9.827 cm) at the end of two years than those in the commercial inoculum treatment (mean = 182.375 ± 9.808 cm), but not significantly larger than plants in the control treatment (mean = 188.942 ± 10.139 cm). Fertilization did not affect plant size ($F_{2,251}$=0.507; P=0.603), however year had a significant effect on plant size ($F_{1,256}$=8.335; P=0.004) as well as season ($F_{1,251}$=4.570; P=0.034).

2.3.2 Reproductive traits

Inoculation significantly affect flowering ($F_{2,112}$=3.736; P=0.027; Figure 2.3), however fertilization treatment ($F_{2,112}$=0.410; P=0.665) and year ($F_{1,123}$=0.089; P=0.766; Figure 2.2) did not. Additionally, plants responded to the inoculation differently in the

two years. Flower production in 2020 was significantly higher in the local inoculum treatment (mean = 200.612 ± 28.814 flowers) compared to plants in the commercial inoculum (mean = 82.399 ± 23.855 flowers), but in 2021 flower production was not significantly different among the inoculum treatments ($F_{2,120}$=6.595; P=0.002; Figure 2.4). Initial plant size had a significant effect on flower production ($F_{1,107}$=5.934; P=0.017) and plants responded differently to the inoculum depending on initial plant size ($F_{2,109}$=3.545; P=0.032; Figure 2.5).

Like flowering, inoculation had a significant effect on the number of berries produced ($F_{2,113}$=3.653; P=0.029; Figure 2.7), but fertilizer treatment ($F_{2,112}$=0.337; P=0.715) and year ($F_{1,125}$=1.660; P=0.120; Figure 2.6) did not. The response of plants to the inoculation treatment differed between the two years such that plants in the local inoculum treatment produced significantly more berries in 2020 (mean = 194.853 ± 26.416 berries) than in 2021 (mean = 82.658 ± 20.899 berries) ($F_{2,122}$=6.947; P=0.001; Figure 2.8). Initial plant size had a significant effect on berry production ($F_{1,106}$=7.047; P=0.009) and plants responded differently to the inoculum depending on initial plant size ($F_{2,107}$=3.320; P=0.040; Figure 2.9).

Fruit set (the proportion of flowers that set fruit) was significantly larger in 2020 (mean = 0.923 ± 0.020) than in 2021 (mean = 0.767 ± 0.019) ($F_{1,137}$=33.483; P<0.0001; Figure 2.10). Inoculation did not significantly affect fruit set ($F_{2,124}$=2.488; P=0.087; Figure 2.11), nor did fertilizer ($F_{2,122}$=0.267; P=0.766) or plant size ($F_{1,123}$=0.559; P=0.456). There were no significant interactions between inoculum, fertilizer, year, or

plant size on the percentage of flowers that set fruit.

Inoculum treatment did not have a significant effect on berry mass ($F_{2,87}$=2.162; P=0.121), nor did fertilizer treatment ($F_{2,88}$=0.055; P=0.947), year ($F_{1,72}$=1.275; P=0.263), or plant size ($F_{1,94}$=0.097; P=0.757). The number of seeds (fertilized ovules) in a berry differed significantly between 2020 and 2021. In 2020, the average number of fertilized ovules per berry was 68.325 ± 1.518 and in 2021 that number significantly increased to 76.302 ± 1.612 ($F_{1,86}$=15.939; P=0.0001; Figure 2.12). However, inoculum treatment did not affect the number of fertilized ovules ($F_{2,96}$=0.211; P=0.810), nor did fertilizer treatment ($F_{2,96}$=0.420; P=0.658) or initial plant size ($F_{1,103}$=0.005; P=0.946).

The sugar content of berries varied significantly between years; in 2020 the average sugar content of a berry (brix) was 11.780 ± 0.208 and in 2021 the average sugar content was 13.272 ± 0.222 ($F_{1,89}$=27.030; P<0.0001; Figure 2.13). The sugar content in berries between fertilizer treatments was marginally significant, and berries from plants receiving half the recommended amount of fertilizer produced sweeter berries than those from the plants receiving no fertilizer ($F_{2,93}$=2.921; P=0.058). Inoculum treatment had no effect on berry sugar content ($F_{2,92}$=0.688; P=0.505), nor did any interactions between inoculum, fertilizer treatment, or year. Plant size did not affect sugar content ($F_{1,101}$=0.189; P=0.664), although plants responded differently to the inoculum depending on initial plant size ($F_{2,95}$=3.210; P=0.045; Figure 2.14).

2.3.3 Potting Media Characteristics

Neither inoculum treatment nor fertilizer had a significant effect on media pH ($F_{2,64}$=1.462; P=0.239; $F_{2,64}$=1.697; P=0.191). There was a significant difference in media pH between years ($F_{1,64}$=54.203; P<0.0001), with potting media in 2021 having a significantly lower pH than in 2020. Both the commercial and local inoculum treatments had a lower pH in 2021 than in 2020 (P=0.0002; P<0.0001), but the control treatment did not significantly differ in pH between years (P=0.259; Table 2.1).

Two forms of nitrogen were assessed: nitrate (NO_3) and ammonium (NH_4). NO_3 did not show any differences between inoculum treatments ($F_{2,66}$=1.447; P=0.243) or fertilizer treatments ($F_{2,66}$=1.801; P=0.173), but there was a significant difference between years, and potting media in 2021 contained significantly less NO_3 than in 2020 ($F_{1,66}$=14.563; P=0.0003; Table 2.1). Inoculum treatment had a marginally significant effect on NH_4 concentration ($F_{2,64}$=3.248; P=0.046), and year had a significant effect ($F_{1,64}$=17.485; P<0.0001; Table 2.1). Fertilizer treatment had no effect on NH_4 concentration ($F_{2,64}$=2.539; P=0.087), and the only significant interaction was inoculum treatment and year. The control treatment in 2020 contained significantly more NH_4 than any other treatment that year and any treatment in 2021 ($F_{2,64}$=8.272; P=0.0006; Table 2.1).

The amount of phosphorus (P) in soils differed significantly by year ($F_{1,64}$=120.553; P<0.0001), with significantly less phosphorus in soils in 2021. Inoculum or fertilizer treatment did not influence phosphorus concentration ($F_{2,64}$=1.369; P=0.262; $F_{2,64}$=0.947; P=0.393; Table 2.1). Plants in the inoculum treatments responded differently

between the two years ($F_{2,64}$=4.18; P=0.016), with all inoculum groups containing less phosphorus in 2021.

The amount of potassium (K) in soils differed significantly between inoculum treatments and years ($F_{2,66}$=6.948; P=0.002; $F_{1,66}$=84.463; P<0.0001), but not between fertilizer treatments ($F_{2,66}$=0.216; P=0.806; Table 2.1). Commercial and local inoculum soils contained significantly more potassium than soils in the control treatment, and soils contained more potassium overall in 2021 compared to 2020. There were no significant interactions among inoculum treatment, fertilizer treatment, or year in potassium content.

2.4 Discussion

Overview

The source of ericoid mycorrhizal fungi affected plant size, number of flowers and berries, the relationship between plant size and sugar content, and the relationship between plant size and flower and berry production. However, the effect of the inoculum was not consistent for all response variables. Plants in the local soil inoculum treatment produced greater numbers of flowers and berries than commercially inoculated plants, while larger plants in the commercial inoculum produced sweeter berries than those in the local inoculum. Although I found differences in inoculation treatment effects between years, I found no effects of fertilizer treatment for any of the response variables measured and no significant interactions between fertilizer and inoculation treatment or between fertilizer and year.

Flower and berry production

Local adaptation in mycorrhizal relationships could enhance plant performance and, in several recent studies, plants inoculated with local fungal taxa outperformed those inoculated with foreign fungi (Klironomos 2003; Taheri and Bever 2011; Pellegrino and Bedini 2014; Middleton et al., 2015; Emam 2016). Here, we found differences in the effects of local versus foreign fungal sources on plant growth and reproductive traits. Plants that received the local soil inoculum were larger and produced more flowers and berries than commercially inoculated plants, especially in 2020. Klironomos (2003) suggests that foreign fungal communities offer less variation in plant response at both ends of the mutualism-parasitism spectrum than locally adapted fungal communities. The considerable difference in the number of flowers and berries produced between years on plants in the local inoculum treatment support the idea of a larger variation in plant response to locally adapted fungal partners. Additionally, soil communities interacting with plant roots can play diverse roles in ecosystem functioning and host plant fitness and can affect introduced species differently than a locally adapted microbe community (Hu et al. 2016).

The relationship between number of flowers and berries produced and plant size differed between inoculum treatments. Plants in the commercial inoculum treatment and control treatment showed a positive correlation between plant size and flower/berry production. However, the production of flowers and berries was statistically independent of plant size for those plants in the local inoculum treatment. This is an interesting result and could be due to various factors. One possible explanation is that as plants grew

larger, they became more pot-bound and could not increase their output any further. Plants in the control and commercial inoculum treatments were consistently smaller and therefore may have had more potential to utilize nutrient, sunlight, and water to increase production of flowers and berries as plants grew bigger.

Many findings to date suggest local soil inoculation out-performs commercial inoculation (Klironomos 2003; Taheri and Bever 2011; Emam 2016), however there is also evidence of increased nutrient acquisition and growth of plants inoculated with a commercial inoculum (Al-Karaki et al. 2004; Chen et al. 2005; Hanane et al. 2020). These findings were discovered in experiments where arbuscular mycorrhizal fungi were used. Perhaps ericoid mycorrhizal fungi interactions with host plants are more specialized and therefore more effective when derived from native soil. Only around 150 ericoid fungal taxa have been found to associate with ericaceous plants, compared to around 1600 different fungal taxa within arbuscular mycorrhizal fungi (Walker et al. 2011; Kivlin et al. 2011; Öpik et al. 2013), suggesting a more specialized relationship between ericaceous plants and ericoid fungal partners.

Fruit set and seed production

In all treatments, fruit set was higher in 2020 than the following year, and the number of seeds per berry was significantly greater in 2021 compared to 2020. Mycorrhizal fungi can have a positive to neutral effect on fertilization and seed development (Bryla and Koide 1990; Ganade and Brown 1997; Gange and Smith 2005; Bona et al. 2017; Liu et al. 2018). Previous results show that inoculation by mycorrhizal

fungi can improve ovule viability (Ghanem et al. 2014). Additionally, mycorrhizal inoculation of wild and cultivated *Lycopersicon esculentum* Mill. increased fruit set and the average number of seeds produced per fruit in some plants, but since all flowers were mechanically pollinated, pollinator visitation did not limit seed production and the most likely difference between inoculated and control plants was nutrient acquisition (Bryla and Koide 1990).

The number of fertilized ovules per berry was larger in 2021 than 2020, possibly due to an increase in successful pollination. Plants invest energy into making the optimal number of ovules and pollen to maximize fertilization and the development of seeds (Gillet and Gregorius 2020). Seed production is usually limited by pollination or resources needed for seed development (Knight et al. 2005; Hove et al. 2016). The most frequent pollinators we observed were bumble bees (unpublished data). Bumble bee rewards such as nectar and pollen may have differed in quality or quantity between years, leading to differences in pollinator visitation and successful pollination.

Sugar content of berries

Inoculation treatment did not have a significant effect on berry sugar content, nor did fertilizer treatment. However, an increase in plant size was negatively correlated with sugar content of berries within inoculated treatments, but positively correlated with berry sweetness in non-inoculated treatments. Mycorrhizal inoculation has significantly increased plant growth (Ważny et al. 2022), nutrient status (Jin et al. 2005; Bati et al. 2015; Zhu et al. 2016), and quality (Castellanos-Morales et al. 2010; Zeng et al. 2014;

Bona et al. 2017) of various crops. An increase in sugar content and fruit quality was observed in citrus plants associating with arbuscular mycorrhizal fungi (Zeng et al. 2014). It is possible that inoculated soil may have had an impact on nutrients transferred between plants and soil microbes, leading to increased carbohydrate transfer to fungal partners and less sugars available for berry sweetness. With a greater carbon allocation to mycorrhizal fungi, less nutrient transfer to host plants could result (Hasselquist et al. 2016). An example of this is explained by past experiments where plants grown in greenhouse environments showed that increased carbon transfer to mycorrhizal fungi resulted in reduced N transfer efficiency, and therefore negatively affected plant growth (Alberton et al. 2007; Alberton and Kupyer 2009).

If soil microbes were taking more than they were giving to the plant, this could have had a negative effect on plant growth, health, and reproduction. With a greater plant size there should be more leaves and surface area for photosynthesis to occur, therefore creating more products of photosynthesis like carbohydrates. Because results of the present study showed an increase in plant size did not lead to an increase in berry sweetness, it is likely to assume that nutrients were being taken away from the plant. Symbiotic interactions between players may shift between mutualism and parasitism depending on internal or external factors (Thompson 1988; Bronstein 1994; Johnson et al. 1997; Hernandez 1998; Herre et al. 1999; Klironomos 2003; Johnson et al. 2003). Neuhauser and Fargione (2004) found that as soil fertility decreases, relative benefits to the plant also decrease and the interaction between fungus and plant could turn parasitic. Johnson et al. (1997) suggested that mycorrhizal associations range from mutualistic to

parasitic depending on the abundance of soil nutrients. Additionally, Johnson et al. (1993) found that parasitism may be more frequent under environmental conditions where soils have been repeatedly fertilized.

Media nutrient concentrations

Nasholm et al. (2013) suggest that fungal nutrient transfer is limited when soils are lacking in nutrients but transfer larger amounts of nutrients when they are readily available. The concentrations of NPK were statistically indistinguishable among fertilizer treatments, but inoculum source significantly affected the amount of NH_4 and K in potting media. Significantly more NH_4 was present in non-inoculated media than those commercially inoculated, potentially because with increased uptake of NH_4 by the plant, there is less left in the potting media. Additionally, inoculated media contained larger amounts of K than non-inoculated controls. As uptake of ammonium increased, there may have been a decrease in uptake of potassium. Intermediate levels of K uptake have been found to facilitate the optimal uptake of nitrogen in apple trees (Xu et al. 2020).

Previous findings suggest that mycorrhizal colonization of ericaceous plant roots is negatively correlated with ammonium in the soil (Scagel 2005; Scagel and Yang 2005), potentially because nutrient availability is high and mycorrhizal assistance is not as needed in these conditions. Roveda-Hoyos et al. (2022) concluded that mycorrhizal inoculation positively affected nutrient uptake in blueberry plants with a nutrient deficiency, reaching optimal levels of N, P, and Ca. There is evidence that mycorrhizal symbiosis can compensate for nutrient limitations, however results from this study did

not corroborate previous findings. The two forms of nitrogen tested, NO_3 and NH_4, convert back and forth frequently due to multiple factors influencing biological activity such as temperature, moisture level, and pH changes (Horneck et al. 2011). The typical amount of ammonium nitrogen in the soil is 2-10 ppm, so the NH_4 level found in our soils (3.4 – 4.5 ppm) is well within that range. Total nitrogen does not equal the sum of NO_3 and NH_4, as nitrogen can be present in the soil in many forms, organic and inorganic, and can very even day to day (Horneck et al. 2011). Additionally, plant available nitrogen is not equivalent to total nitrogen because NH_4 is what is up taken by blueberry plants, and pH needs to stay low (acidic) to keep nitrogen in this form (Ames et al. 1983; Peterson et al. 1988).

Phosphorus is one of the critical nutrients provided by mycorrhizal fungi, and up to 80% of P can be supplied by fungal symbionts, extenuating the importance of this interaction (van der Heijden 2017). Healthy levels of phosphorus in soil range from 25 – 50 ppm (Bruulsema 2006), and soil tests from both years show phosphorus concentrations in or exceeding this range. Previous research has stated that root colonization by mycorrhizal fungi decreased as P availability increased in the soil medium (Koide et al. 1999). In addition to phosphorus, Potassium levels were in the high range (250 – 800 ppm), but not excessive (Horneck et al. 2011). Potassium levels in our soils were around 300 – 400 ppm, and inoculated soils, regardless of source, contained more K than the non-inoculated control. Because nutrients were present in all fertilizer treatments within the optimum range, it is likely that nutrients were not limiting in these soils. In a past study using ericaceous container-grown plants, root colonization did not

differ between plants receiving varying amounts of fertilizer, indicating that nutrient levels may have been high enough to suppress mycorrhizal associations (Zinati et al. 2011).

Caveats

Being confined to a pot can alter a plant's physiology and growth, as well as limit its root system and interactions with microbes in soils. Different additives in soil, such as perlite and vermiculite used in the media mix in the present study, can also modify soil nutrient availability (Dalling et al. 2013). A meta-analysis of the effect of pot size on plant growth revealed that doubling pot size increased plant biomass production by 43%, and about 65% of studies using pots in current research practice are using pots that are smaller than the recommended pot size-plant biomass ratio (1gL-1) (Poorter et al. 2012a).

At the conclusion of this experiment, these plants had been confined to pots for 4 years, starting in the spring of 2018. As their above-ground biomass increased, their root system likely increased as well, and were unable to expand to their full potential of area in the soil. Because plants in the local inoculum treatment were consistently larger than commercially inoculated and non-inoculated plants, plants in the local inoculum treatment may have been most negatively impacted by being pot-bound. Similarly, microbe partners in the soil may not have been able to assist the plant as much if they were also constrained by soil area and nutrients available. Additionally, inoculation at sowing showed increased colonization compared to inoculation at transplanting, and a negative effect on shoot growth with increased inoculum and P concentration. Negative

effects on shoot growth after inoculation have also been observed (Biermann and Linderman 1983).

Another important factor to note is the extreme weather that may have influenced fall bud formation and overwintering in the fall of 2020. In November of 2020, there was a period of extremely low freezing temperatures (NEWA, Cornell University) before plants had undergone cold hardening. This could have damaged the formation of buds, therefore explaining the decrease in flowering the next summer. Stems may have also suffered from frost damage, dying, and falling off leading to a decrease in stem height if these stems were not able to continue growing the next spring. These external environmental factors may have played a role in the variations we saw in flower and berry production as well as plant size decreases between years.

Conclusion

In conclusion, mycorrhizal inoculation affected growth and reproductive traits in highbush blueberry. The local soil inoculum had a positive effect on plant growth as well as the number of flowers and berries produced, while the commercial inoculum did not. The local soil and commercial inoculum varied in their effects, however both inoculums had a negative relationship between plant size and berry sugar content, suggesting a potential role of plant-fungal interactions in available carbohydrates. Plant response to inoculation varied year to year, most noticeably in the local inoculum treatment, while growth and reproductive traits of commercially inoculated plants remained more stable. There seemed to be no effect of inoculation in limited fertilizer conditions, and nutrient

levels were optimal and consistent across all fertilizer treatments. Further work must be done to test the ability of ericoid mycorrhizal fungi to compensate for limited fertilizer application and the potential for reduced fertilizer use in agricultural settings.

2.5 Table Legend

Table 2.1: Average media pH, ± 1 standard error, and average nutrient concentrations (mg/kg or ppm), ± 1 standard error, in each of the inoculum, fertilizer, and year treatments. Inoculum treatments include non-inoculated control, commercial inoculum, and local inoculum. Fertilizer treatments include the recommended amount of fertilizer, half of the recommended amount, and no fertilizer. In 2020 and 2021, soil samples were taken seven weeks after fertilization.

Table 2.1: Average media pH, ± 1 standard error, and average nutrient concentrations

Treatment	Media pH	NO_3	NH_4	P	K	Mg	Ca	Fe	S
Control	6.390 ± 0.054	0.472 ± 0.114	4.259 ± 0.229	66.313 ± 3.893	299.764± 19.408	1052.533 ± 30.708	6416.094 ± 146.557	4.980 ± 0.252	19.568 ± 1.652
Commercial	6.394 ± 0.054	0.497 ± 0.114	3.483 ± 0.229	70.102 ± 3.893	397.994 ± 19.408	987.356 ± 30.708	6096.756 ± 146.557	5.141 ± 0.252	19.109 ± 1.652
Local	6.465 ± 0.054	0.718 ± 0.114	4.118 ± 0.229	75.388 ± 3.893	369.931 ± 19.408	1080.333 ± 30.708	6490.739 ± 146.557	5.251 ± 0.252	20.901 ± 1.652
None	6.419 ± 0.054	0.397 ± 0.114	3.753 ± 0.224	68.466 ± 4.006	345.973 ± 19.408	1083.560 ± 30.062	6485.314 ± 143.471	5.235 ± 0.247	16.867 ± 1.688
Half	6.348 ± 0.054	0.693 ± 0.114	4.362 ± 0.224	68.497 ± 4.006	363.264 ± 19.408	994.539 ± 30.062	6117.585 ± 143.471	5.206 ± 0.247	18.544 ± 1.688
Full	6.481 ± 0.054	0.597 ± 0.114	3.745 ± 0.224	74.841 ± 4.006	358.452 ± 19.408	1042.123 ± 30.062	6400.689 ± 143.471	4.931 ± 0.247	24.167 ± 1.688
2020	6.641 ± 0.050	0.819 ± 0.106	3.400 ± 0.209	95.296 ± 3.553	250.704 ± 18.098	1207.815 ± 28.033	6576.704 ± 133.788	5.748 ± 0.230	28.296 ± 1.541
2021	6.191 ± 0.039	0.307 ± 0.082	4.507 ± 0.162	45.906 ± 2.752	461.089 ± 14.018	872.333 ± 21.714	6092.356 ± 103.632	4.500 ± 0.178	11.422 ± 1.193

(mg/kg or ppm), ± 1 standard error, in each of the inoculum, fertilizer, and year treatments.

2.6 Figure Legend

Figure 2.1: The mean plant size (cm) in control, commercial inoculum, and local inoculum treatments over time. Size measurements were taken in the spring and fall of 2020 and 2021. Inoculum treatment had a significant effect on plant size ($F_{2,161}$=3.157; P=0.045), and the local inoculum plants were overall larger than plants in the commercial inoculum treatment.

Figure 2.2: The mean number of flowers produced, ± 1 standard error, on an individual plant in 2020 and 2021. The average number of flowers produced was not significantly different between years ($F_{1,125}$=0.118; P=0.732).

Figure 2.3: The mean number of flowers produced, ± 1 standard error, in control, commercial inoculum, and local soil inoculum treatments. Inoculum source significantly affected flowering ($F_{2,112}$=3.736; P=0.027). This analysis and figure include flower counts from 2020 and 2021.

Figure 2.4: The mean number of flowers produced, ± 1 standard error, in control, commercial inoculum, and local soil inoculum treatments in 2020 and 2021. A Least-Squares Means Tukey HSD Test was used to test the interaction between inoculum treatment and year. The local inoculum treatment produced significantly more flowers than the control and commercial inoculum treatments in 2020, but not in 2021 ($F_{2,120}$=6.595; P=0.002).

Figure 2.5: The correlation between plant size (cm) and the number of flowers produced in control, commercial inoculum, and local inoculum treatments. The interaction between plant size and the number of flowers produced is shown. Plants respond to the commercial inoculum and local inoculum differently depending on initial plant size ($F_{2,107}$=3.636; P=0.030).

Figure 2.6: The mean number of berries produced, ± 1 standard error, on an individual plant in 2020 and 2021. The average number of berries produced was not significantly different between years ($F_{1,125}$=0.118; P=0.732).

Figure 2.7: The mean number of berries, ± 1 standard error, produced in control, commercial inoculum, and local soil inoculum treatments. Inoculum source significantly affected berry production ($F_{2,113}$=3.653; P=0.029). This analysis and figure include berry counts from 2020 and 2021.

Figure 2.8: The mean number of berries produced, ± 1 standard error, in control, commercial inoculum, and local soil inoculum treatments in 2020 and 2021. A Least-Squares Means Tukey HSD Test was used to test the interaction between inoculum treatment and year. The local inoculum treatment produced significantly more berries than the control and commercial inoculum treatments in 2020, but not in 2021 ($F_{2,122}$=6.947; P=0.001).

Figure 2.9: The correlation between plant size (cm) and the number of berries produced in control, commercial inoculum, and local inoculum treatments. The interaction between plant size and the number of berries produced is shown. Plants respond to the commercial inoculum and local inoculum differently depending on initial plant size ($F_{2,107}$=3.320; P=0.040).

Figure 2.10: The mean fruit set (the proportion of flowers that set fruit), ± 1 standard error, on an individual plant in 2020 and 2021. The average fruit set significantly differed between years ($F_{1,137}$=33.483; P<0.0001).

Figure 2.11: The average fruit set, ± 1 standard error, in control, commercial inoculum, and local soil inoculum treatments in 2020 and 2021. Fruit set was significantly larger in 2020 (0.923 ± 0.020) than in 2021 (0.767 ± 0.019) across all inoculum treatments ($F_{1,137}$=33.483; P<0.0001).

Figure 2.12: A boxplot showing the median, 1st quartile, 3rd quartile, minimum, and maximum number of seeds (fertilized ovules) in 2020 and 2021. Significantly more fertilized ovules were counted in 2021 (76.302 ± 1.612) compared to 2020 (68.325 ± 1.518; $F_{1,86}$=15.939; P=0.0001). Outliers are shown as data points.

Figure 2.13: A boxplot showing the median, 1st quartile, 3rd quartile, minimum, and maximum sugar content of berries (brix) in 2020 and 2021. Berries were significantly sweeter in 2021 (13.272 ± 0.222) compared to 2020 (11.780 ± 0.208; $F_{1,89}$=27.030;

P<0.0001).

Figure 2.14: The correlation between plant size (cm) and sugar content in berries (brix) in control, commercial inoculum, and local inoculum treatments. The interaction between plant size and berry sweetness is shown. Inoculated plants show a negative correlation between plant size and sugar content, while control plants show a positive correlation between plant size and sugar content ($F_{2,95}$=3.210; P=0.045).

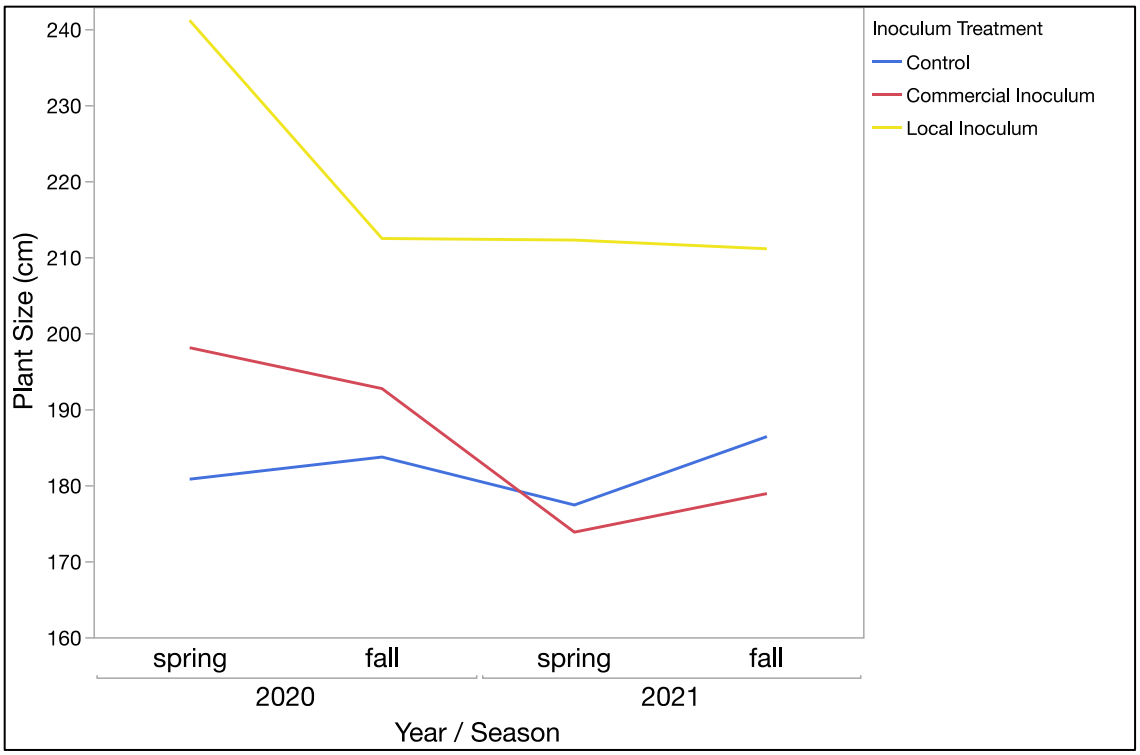

Figure 2.1: The mean plant size (cm) in control, commercial inoculum, and local inoculum treatments over time. Size measurements were taken in the spring and fall of 2020 and 2021. Inoculum treatment had a significant effect on plant size ($F_{2,161}$=3.157; P=0.045).

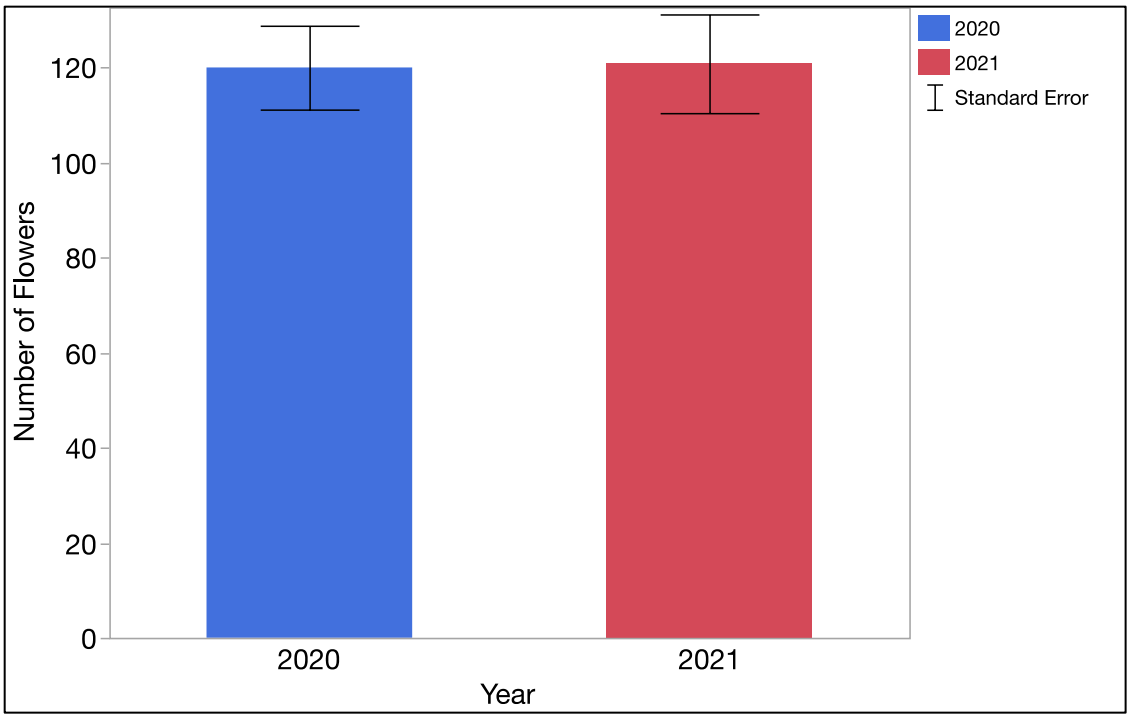

Figure 2.2: The mean number of flowers produced, ± 1 standard error, on an individual plant in 2020 and 2021. The average number of flowers produced was not significantly different between years ($F_{1,125}$=0.118; P=0.732).

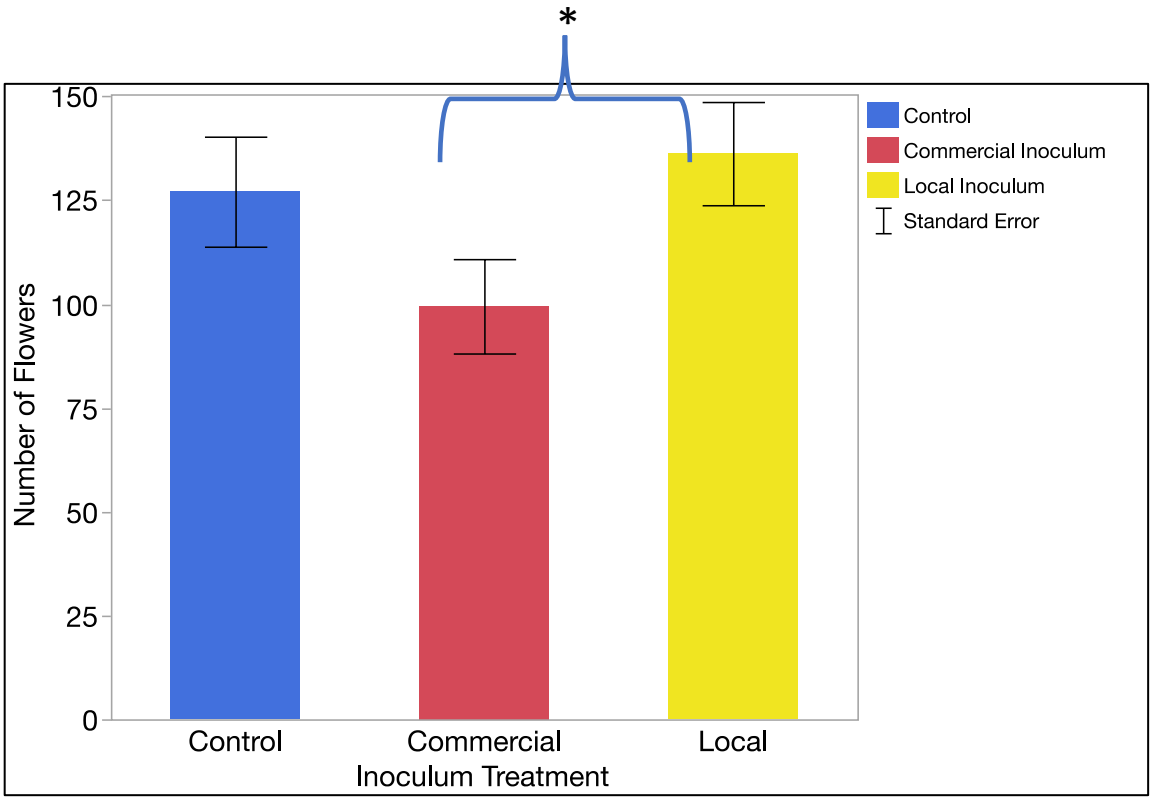

Figure 2.3: The mean number of flowers produced, ± 1 standard error, in control, commercial inoculum, and local soil inoculum treatments. Inoculum source significantly affected flowering ($F_{2,112}$=3.736; P=0.027). This analysis and figure include flower counts from 2020 and 2021.

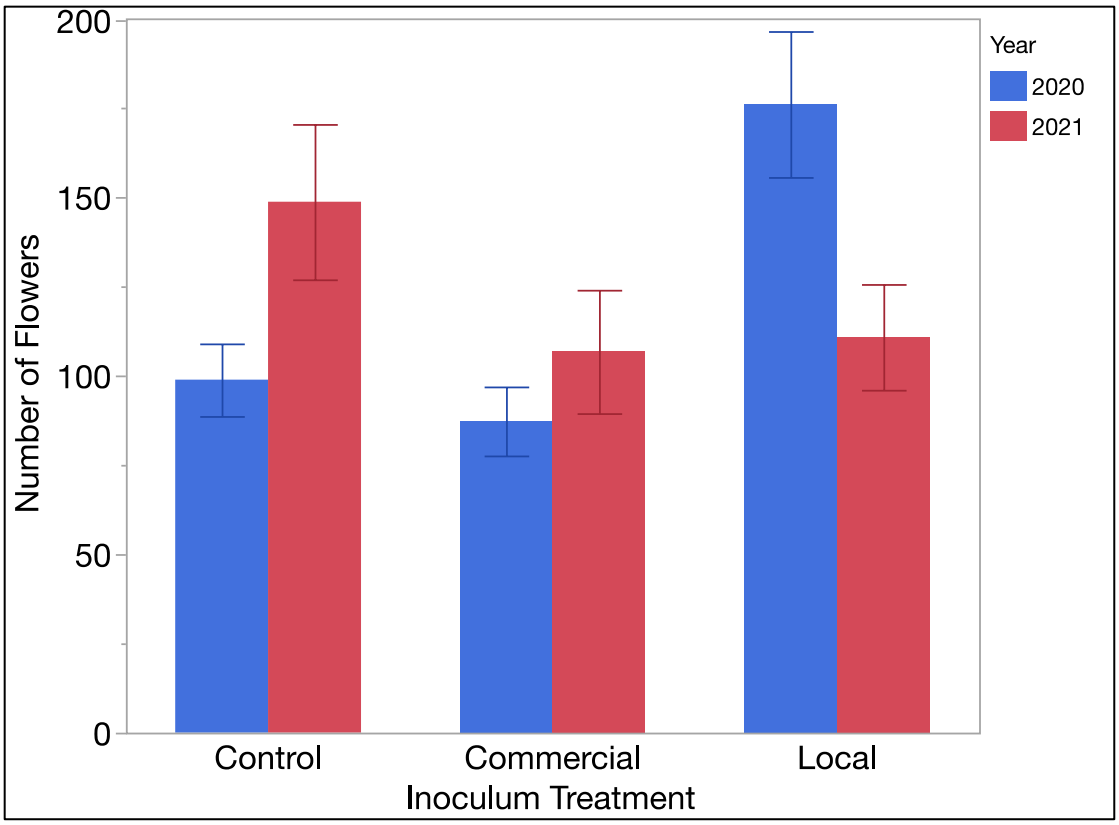

Figure 2.4: The mean number of flowers produced, ± 1 standard error, in control, commercial inoculum, and local soil inoculum treatments in 2020 and 2021. A Least-Squares Means Tukey HSD Test was used to test the interaction between inoculum treatment and year. The local inoculum treatment produced significantly more flowers than the control and commercial inoculum treatments in 2020, but not in 2021 ($F_{2,120}$=6.595; P=0.002).

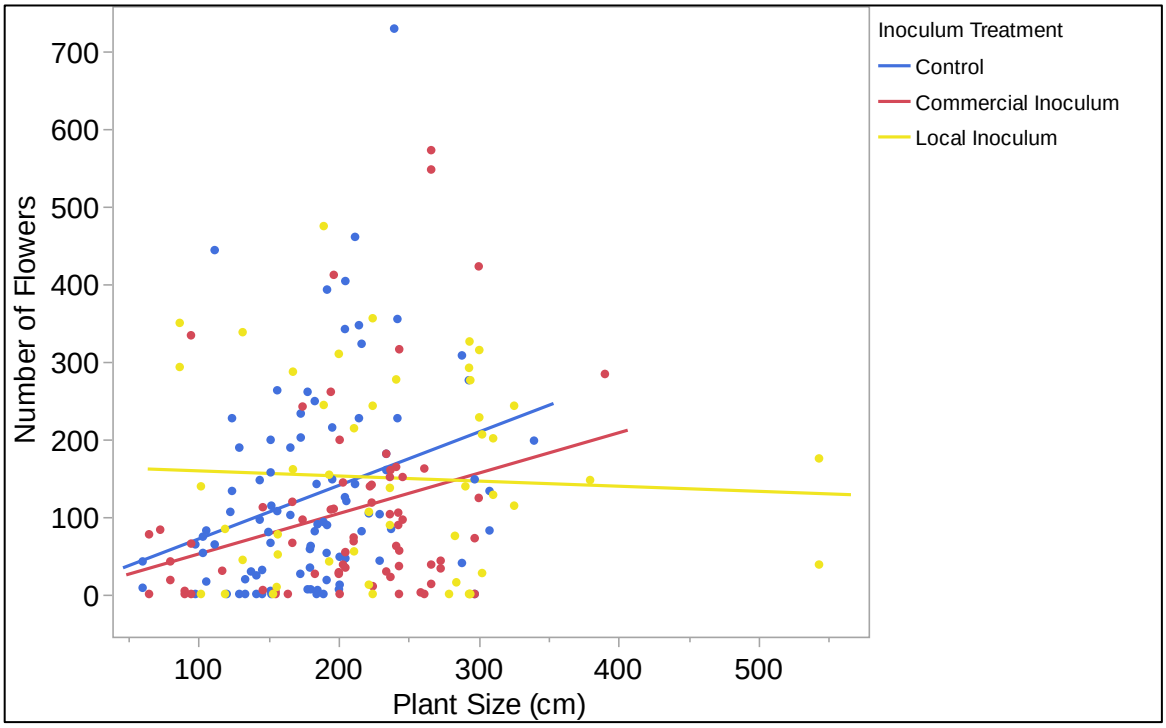

Figure 2.5: The correlation between plant size (cm) and the number of flowers produced in control, commercial inoculum, and local inoculum treatments. The interaction between plant size and the number of flowers produced is shown. Plants respond to the commercial inoculum and local inoculum differently depending on initial plant size ($F_{2,107}$=3.636; P=0.030).

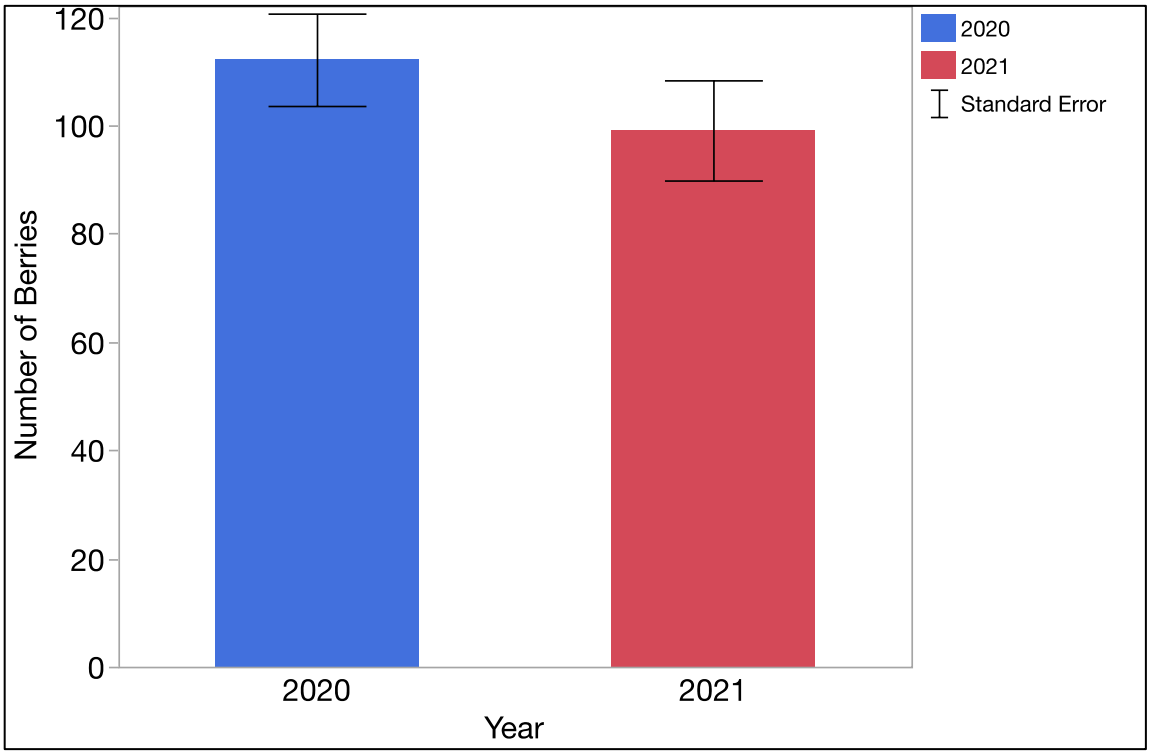

Figure 2.6: The mean number of berries produced, ± 1 standard error, on an individual plant in 2020 and 2021. The average number of berries produced was not significantly different between years ($F_{1,125}$=0.118; P=0.732).

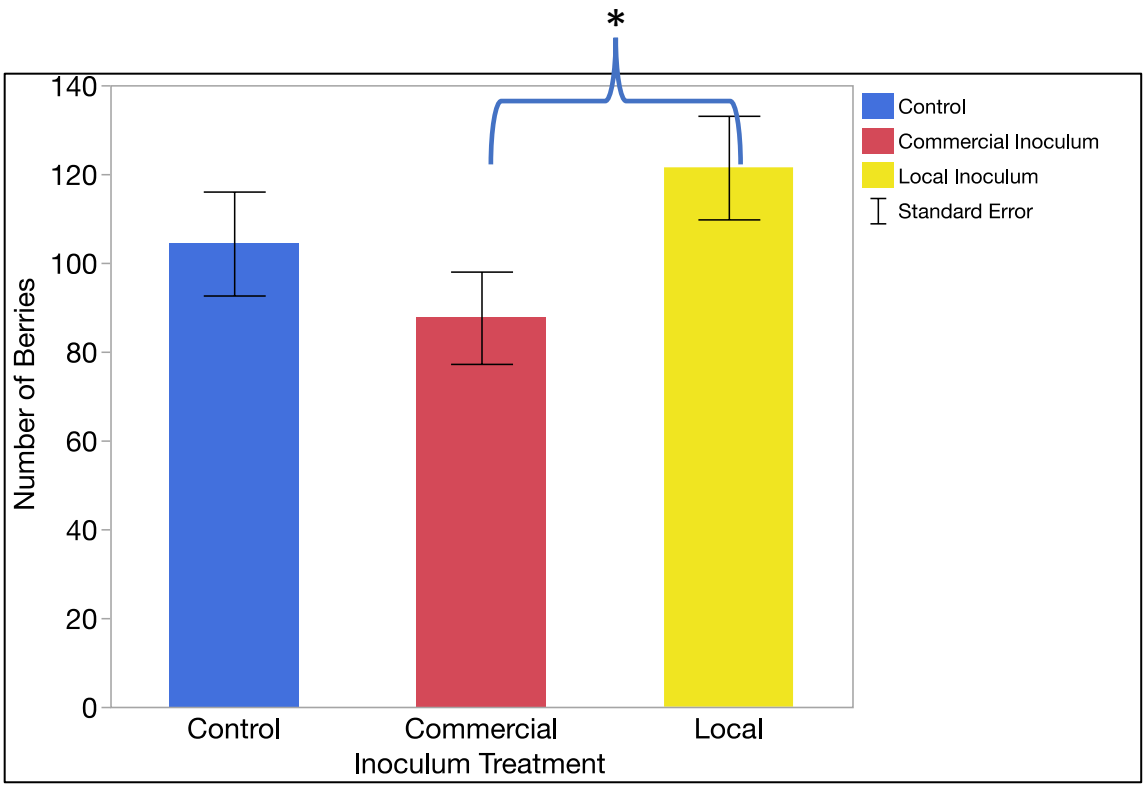

Figure 2.7: The mean number of berries, ± 1 standard error, produced in control, commercial inoculum, and local soil inoculum treatments. Inoculum source significantly affected berry production ($F_{2,113}$=3.653; P=0.029). This analysis and figure include berry counts from 2020 and 2021.

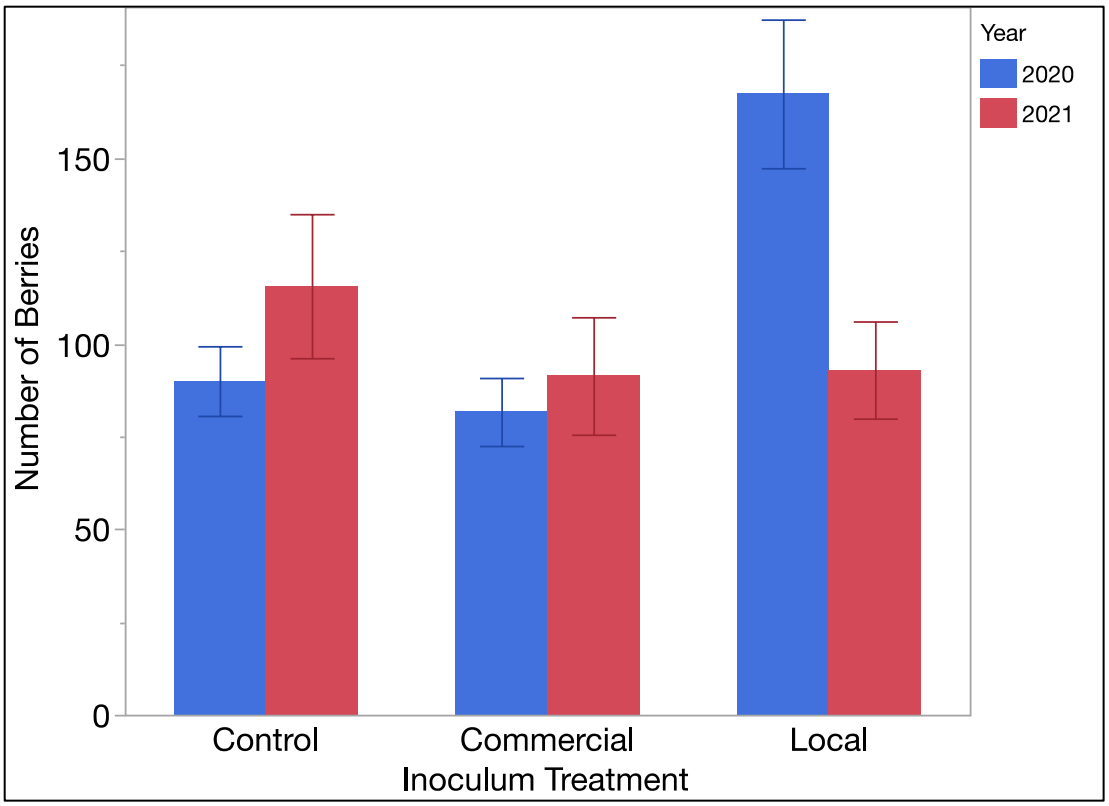

Figure 2.8: The mean number of berries produced, ± 1 standard error, in control, commercial inoculum, and local soil inoculum treatments in 2020 and 2021. A Least-Squares Means Tukey HSD Test was used to test the interaction between inoculum treatment and year. The local inoculum treatment produced significantly more berries than the control and commercial inoculum treatments in 2020, but not in 2021 ($F_{2,122}$=6.947; P=0.001).

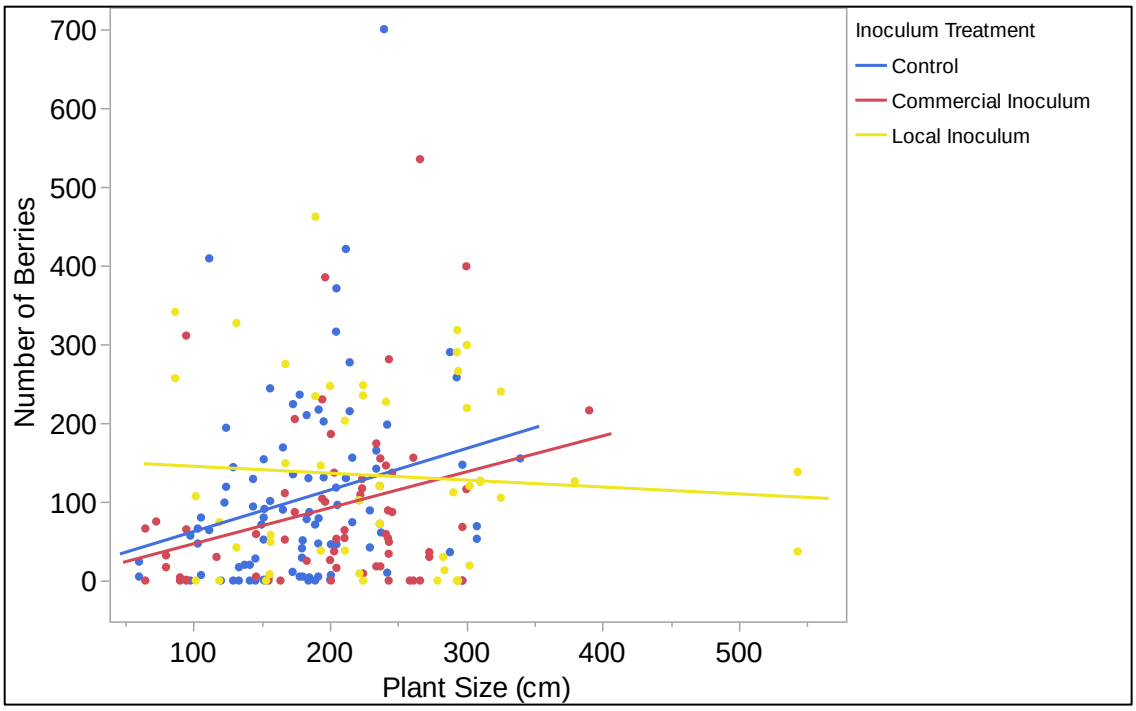

Figure 2.9: The correlation between plant size (cm) and the number of berries produced in control, commercial inoculum, and local inoculum treatments. The interaction between plant size and the number of berries produced is shown. Plants respond to the commercial inoculum and local inoculum differently depending on initial plant size ($F_{2,107}$=3.320; P=0.040).

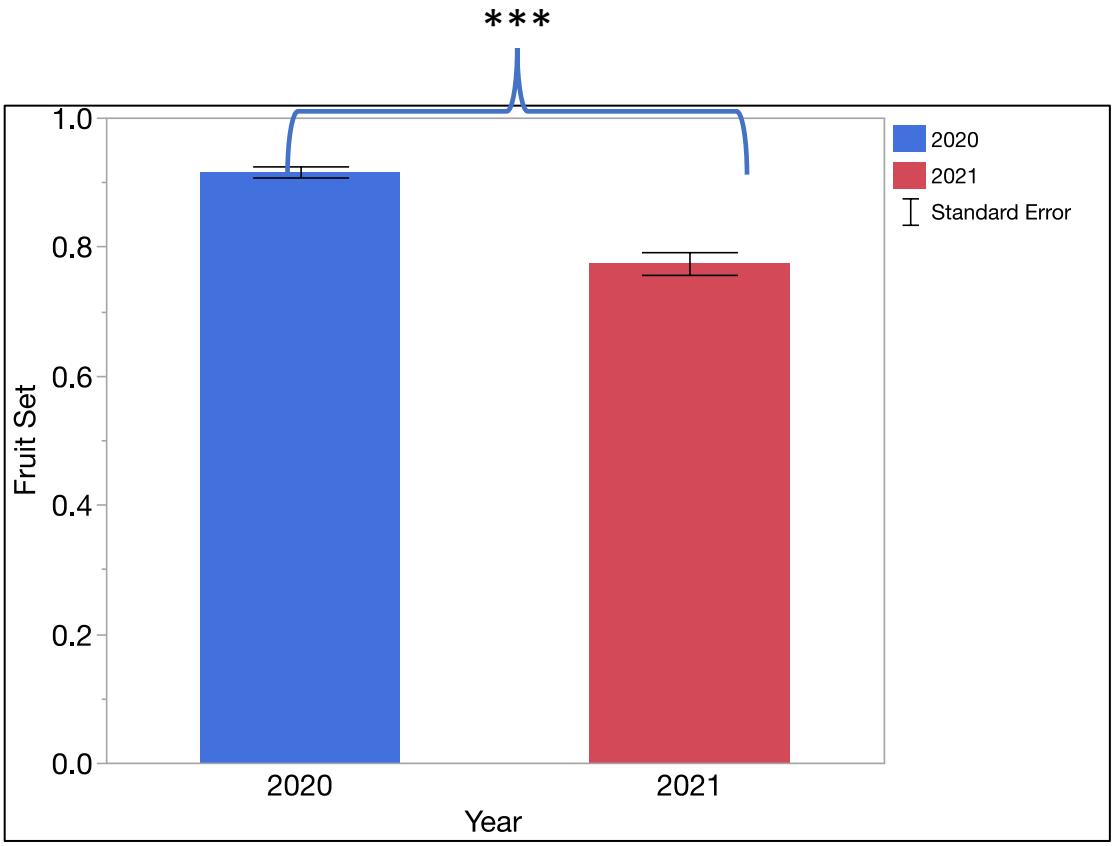

Figure 2.10: The mean fruit set (the proportion of flowers that set fruit), ± 1 standard error, on an individual plant in 2020 and 2021. The average fruit set significantly differed between years ($F_{1,137}$=33.483; P<0.0001).

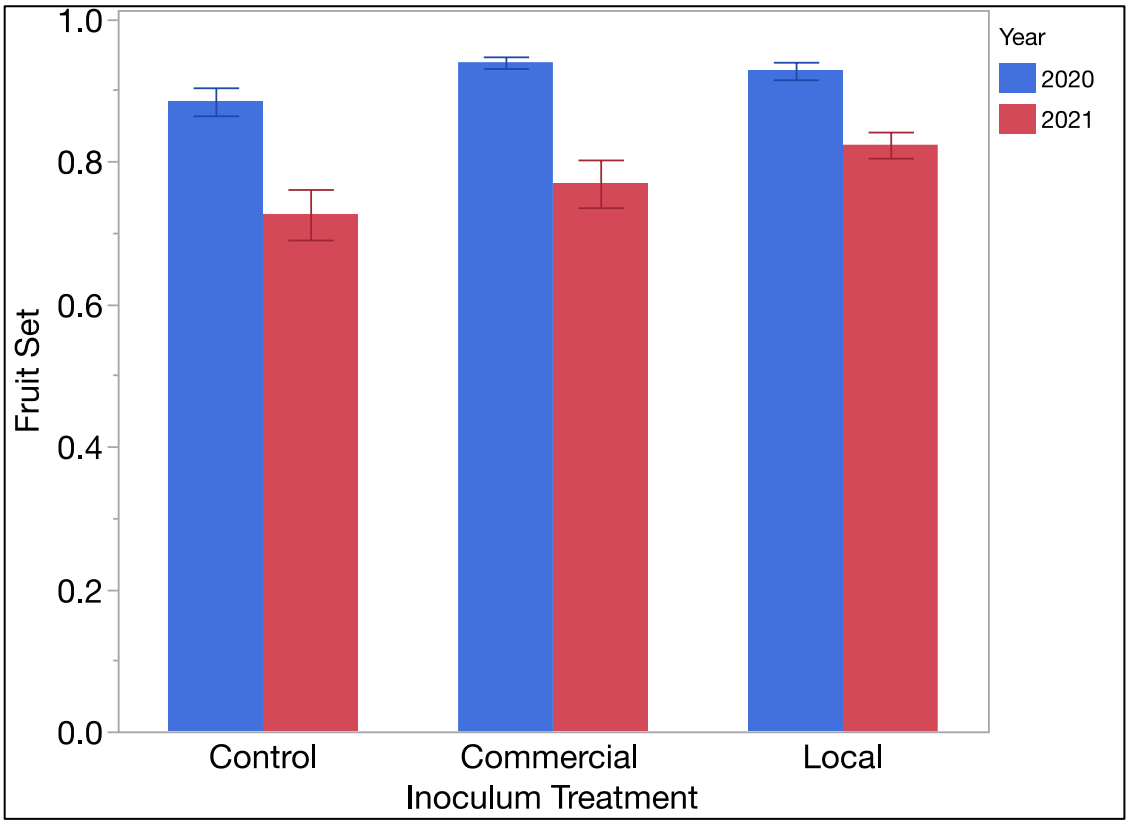

Figure 2.11: The average fruit set, ± 1 standard error, in control, commercial inoculum, and local soil inoculum treatments in 2020 and 2021. Fruit set was significantly larger in 2020 (0.923 ± 0.020) than in 2021 (0.767 ± 0.019) across all inoculum treatments ($F_{1,137}$=33.483; P<0.0001).

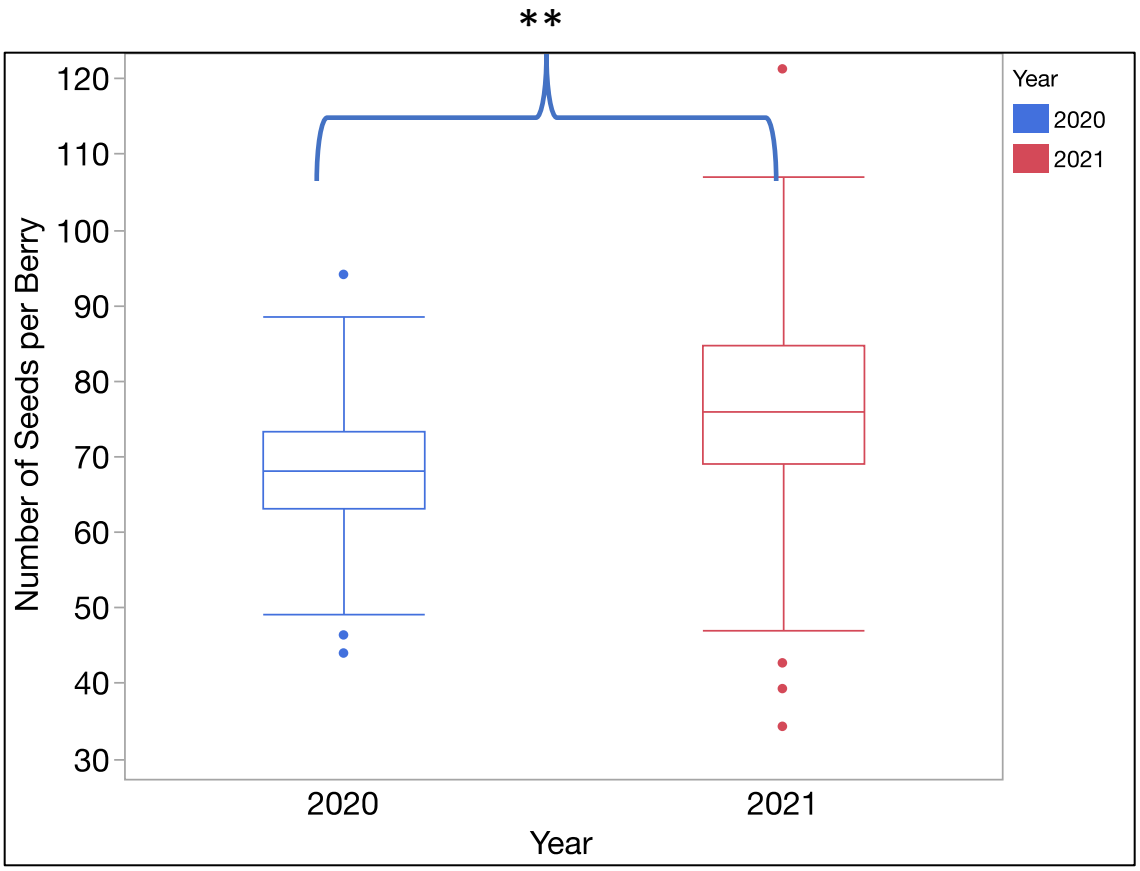

Figure 2.12: A boxplot showing the median, 1st quartile, 3rd quartile, minimum, and maximum number of seeds (fertilized ovules) in 2020 and 2021. Significantly more fertilized ovules were counted in 2021 (76.302 ± 1.612) compared to 2020 (68.325 ± 1.518; $F_{1,86}$=15.939; P=0.0001). Outliers are shown as data points.

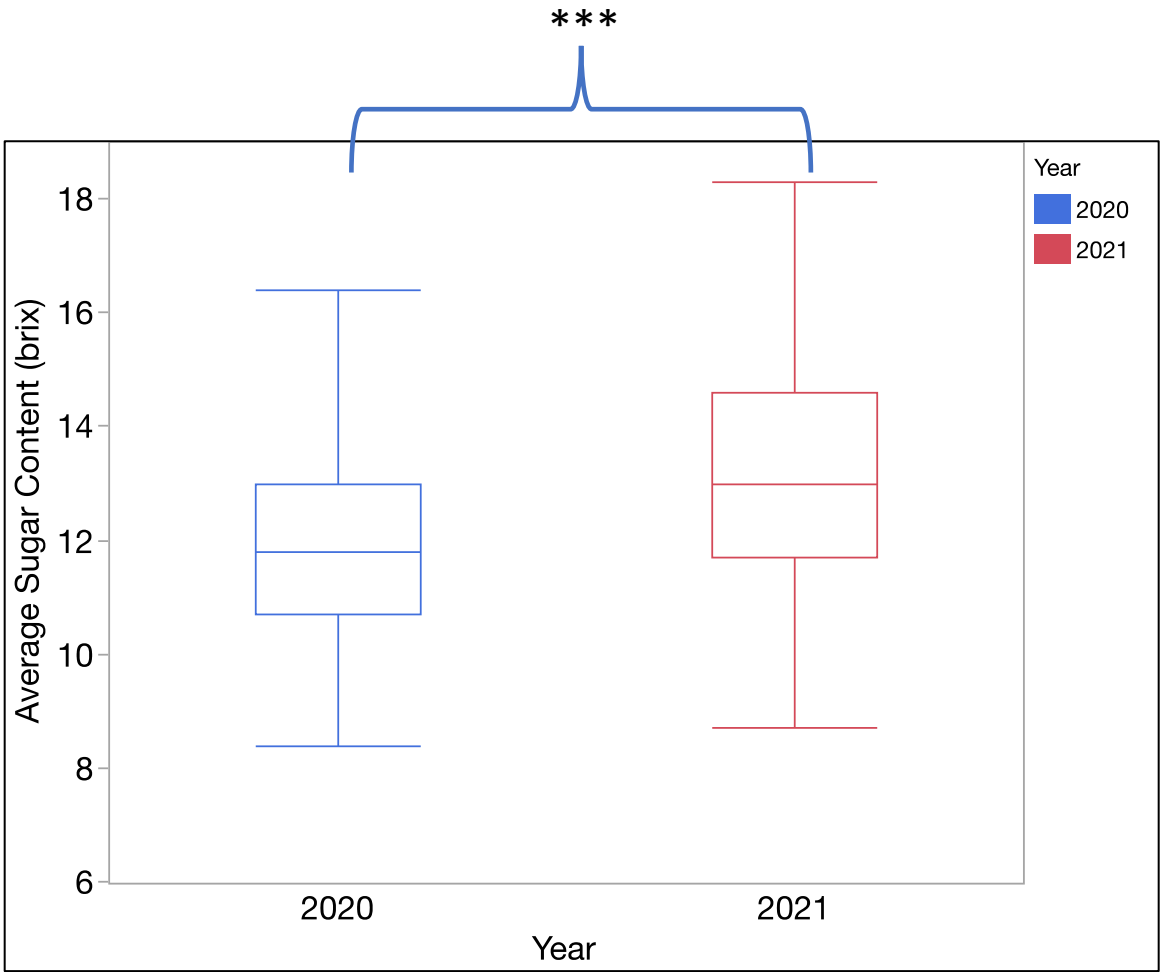

Figure 2.13: A boxplot showing the median, 1st quartile, 3rd quartile, minimum, and maximum sugar content of berries (brix) in 2020 and 2021. Berries were significantly sweeter in 2021 (13.272 ± 0.222) compared to 2020 (11.780 ± 0.208; $F_{1,89}$=27.030; P<0.0001).

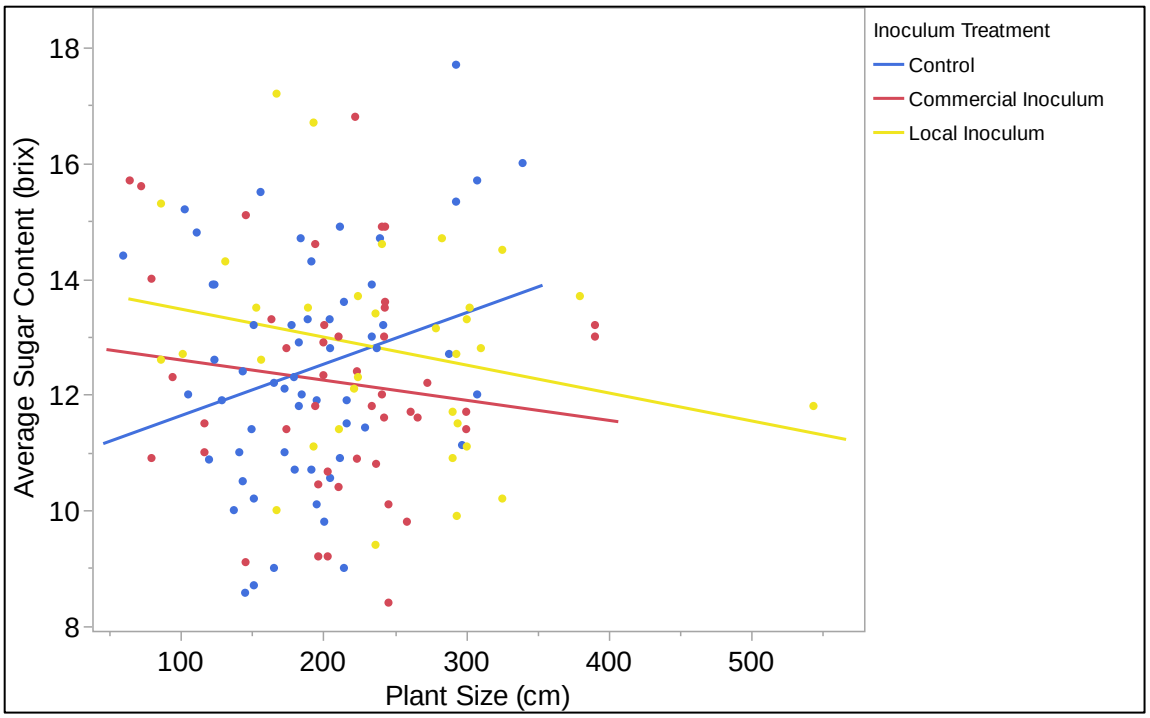

Figure 2.14: The correlation between plant size (cm) and sugar content in berries (brix) in control, commercial inoculum, and local inoculum treatments. The interaction between plant size and berry sweetness is shown. Inoculated plants show a negative correlation between plant size and sugar content, while control plants show a positive correlation between plant size and sugar content ($F_{2,95}$=3.210; P=0.045).

2.7 Works Cited

Alberton, O., and T. W. Kuyper. 2009. Ectomycorrhizal fungi associated with *Pinus sylvestris* seedlings respond differently to increased carbon and nitrogen availability: implications for ecosystem responses to global change. Global Change Biology, 15: 166– 175.

Alberton, O., T. W. Kuyper, and A. Gorissen. 2007. Competition for nitrogen between *Pinus sylvestris* and ectomycorrhizal fungi generates potential for negative feedback under elevated CO_2. Plant and Soil, 296: 159– 172.

Al-Karaki, G., McMichael, B., and Zak, J. 2004. Field response of wheat to arbuscular mycorrhizal fungi and drought stress. Mycorrhiza. 14: 263-269.

Al-Maliki, Salwan, and Mugtaba AL-Masoudi. 2018. Interactions between Mycorrhizal Fungi, Tea Wastes, and Algal Biomass Affecting the Microbial Community, Soil Structure, and Alleviating of Salinity Stress in Corn Yield (*Zea mays* L.). Plants 7: 63.

Ames, R.N., Reid, C.P.P., Porter, L.K., Cambardella, C. 1983. Hyphal uptake and transport of nitrogen from two 15N labelled sources by *Glomus mosseae*, a vesicular arbuscular mycorrhizal fungus. New Phytologist, 95: 381–396.

Barea J.M., Azcón-Aguilar C., Azcón R. 1987. Vesicular-arbuscular mycorrhiza improve both symbiotic N2 fixation and N uptake from soil as assessed with a 15N technique under field conditions. New Phytologist, 106: 717–725.

Bati, C. B., E. Santilli, and L. Lombardo. 2015. Effect of arbuscular mycorrhizal fungi on growth and on micronutrient and macronutrient uptake and allocation in olive plantlets growing under high total Mn levels. Mycorrhiza, 25: 97–108.

Bell TL, Pate JS. 1996. Nitrogen and phosphorus nutrition in mycorrhizal Epacridaceae of South-west Australia. Annals of Botany 77: 389 – 397.

Biermann, B., and R.G. Linderman. 1983. Effect of container plant growth medium and fertilizer phosphorus on establishment and host growth response to vesicular-arbuscular mycorrhizae. Journal of the American Society of Horticulture Science, 108: 962–971.

Bona, E., S. Cantamessa, N. Massa, P. Manassero, F. Marsano, A. Copetta, et al. 2017. Arbuscular mycorrhizal fungi and plant growth-promoting pseudomonads improve yield, quality, and nutritional value of tomato: a field study. Mycorrhiza, 27: 1–C11.

Bronstein, J.L. 1994. Conditional outcomes in mutualistic interactions. Trends in Ecology and Evolution, 9: 214-217.

Brody, A. K., B. Waterman, T. H. Ricketts, A. L. Degrassi, J. B. Gonzalez, J. M. Harris,

and L. L. Richardson. 2019. Genotype-specific effects of ericoid mycorrhizae on floral traits and reproduction in *Vaccinium corymbosum*. Botany, 106: 1412-1422.

Bruulsema, T.W. 2006. Soil Fertility in the Northeast Region. Better Crops, 90: 8-10.

Bryla, D.R. and R.T. Koide. 1990. Regulation of reproduction in wild and cultivated *Lycopersicon esculentum* Mill. by vesicular-arbuscular mycorrhizal infection. Oecologia, 84: 74-81.

Cairney, J. W. G. and A.A. Meharg. 2003. Ericoid mycorrhiza: A partnership that exploits harsh edaphic conditions. European Journal of Soil Science, 54: 735-740.

Castellanos-Morales, V., J. Villegas, S. Wendelin, H. Vierheiling, R. Eder, and R. Cardenas-Navarro. 2010. Root colonization by the arbuscular mycorrhizal fungus Glomus intraradices alters the quality of strawberry fruit (*Fragaria ananassa* Duch.) at different nitrogen levels. Journal of the Science of Food and Agriculture, 90: 1774–1782.

Cavagnaro TR, Gao L-L, Smith FA, Smith SE. 2001. Morphology of arbuscular mycorrhizas is influenced by fungal identity. New Phytologist, 151: 469–75

Chen, X., Wu, CH., Tang, JJ., and Hu, SJ. 2005. Arbuscular mycorrhizae enhance metal lead uptake and growth of host plants under a sand culture experiment. Chemosphere, 60: 665-671.

Dalling, J.W., Winter, K., Andersen, K.M. et al. 2013. Artefacts of the pot environment on soil nutrient availability: implications for the interpretation of ecological studies. Plant Ecology, 214: 329–338.

Douglas, A.E. 2010. The Symbiotic Habit. Princeton University Press, Princeton, NJ. Mutualisms.

Emam, T. 2016. Local soil, but not commercial AMF inoculum, increases native and non-native grass growth at a mine restoration site. Restoration Ecology, 24: 35-44.

Frew, A. 2020. Contrasting effects of commercial and native arbuscular mycorrhizal fungal inoculants on plant biomass allocation, nutrients, and phenolics. Plants, People, Planet, 00: 1– 5.

Ganade, G. and V.K. Brown. 1997. Effects of below-ground insects, mycorrhizal fungi and soil fertility on the establishment of *Vicia* in grassland communities. Oecologia, 109: 374-381.

Gange, A.C. and Smith, A.K. 2005. Arbuscular mycorrhizal fungi influence visitation rates of pollinating insects. Ecological Entomology, 30: 600-606.

George E, Marschner H, Jakobsen I. 1995. Role of arbuscular mycorrizal fungi in uptake of phosphorus and nitrogen from soil. Critical Reviews in Biotechnology, 15: 257–70.

Ghanem, G., A. Ewald, S. Zerche, and F. Hennig. 2014. Effect of root colonization with *Piriformospora indica* and phosphate availability on the growth and reproductive biology of a *Cyclamen persicum* cultivar. Scientia Horticulturae, 172: 233-241.

Gillet, E.M. and H.R. Gregorius. 2020. Effects of reproductive resource allocation and pollen density on fertilization success in plants. BMC Ecology, 20: 26.

Giri, B., Kapoor, R., and Mukerji, KG. 2007. Improved tolerance of *Acacia nilotica* to salt stress by arbuscular mycorrhiza, *Glomus fasciculatum* may be partly related to elevated K/Na ratios in root and shoot tissues. Microbial Ecology, 54: 753-760.

Grelet, G.A., Johnson, D., Paterson, E., Anderson, I.C., Alexander, I.J. 2009. Reciprocal carbon and nitrogen transfer between an ericaceous dwarf shrub and fungi isolated from *Piceirhiza bicolorata* ectomycorrhizas. New Phytologist, 182: 359-366.

Hanane, B., Abdelilah, M., Said, W., Abdelmajid, M., Zainab, ET., Allal, D., Abdelkarim, FM., and Cherkaoui, E. 2020. Improvement of growth and development of olive tree by mycorrhizal autochthonous inoculum. Research Journal of Biotechnology, 15: 76-84.

Harder L.D.**,** S.A. Richards, and M.B. Routley**.** 2008**.** Effects of reproductive compensation, gamete discounting and reproductive assurance on mating-system diversity in hermaphrodites. Evolution, 62: 157–72.

Hasselquist, N.J., Metcalfe, D.B., Inselsbacher, E., Stangl, Z., Oren, R., Näsholm, T. and Högberg, P. 2016. Greater carbon allocation to mycorrhizal fungi reduces tree nitrogen uptake in a boreal forest. Ecology, 97: 1012-1022.

Hawkins HJ, Johansen A, George E. 2000. Uptake and transport of organic and inorganic nitrogen by arbuscular mycorrhizal fungi. Plant and Soil, 226: 275–85.

Hernandez, M.J. 1998. Dynamics of transitions between population interactions: A non-linear interaction a-function defined. Proceedings of the Royal Society B: Biological Sciences, 265: 1404.

Herre, E.A., N. Knowlton, U.G. Mueller, S.A. Rehner. 1999. The evolution of mutualisms: exploring the paths between conflict and cooperation. Trends in Ecology & Evolution, 14: 49-53.

Hobbie EA, Hoff CJ, Bryce JG, Colpaert JV, Hallett RA. 2009. Nutrient supply rate and mycorrhizal colonization control patterns of element distribution in ectomycorrhizal pine. Communications in Soil Science and Plant Analysis, 40: 3503–3523.

Hodge A, Storer K. 2015. Arbuscular mycorrhiza and nitrogen: implications for individual plants through to ecosystems. Plant and Soil, 386: 1–19.

Horneck D.A., D.M. Sullivan, J.S. Owen, and J.M. Hart. 2011. Soil Test Interpretation Guide. Oregon State University.

Hove A.A., S.J. Mazer, and C.T. Ivey. 2016. Seed set variation in wild *Clarkia* populations: teasing apart the effects of seasonal resource depletion, pollen quality, and pollen quantity. Ecology and Evolution, 6: 6524–36.

Hu, J., Z. Wei, V.P. Friman, S.H. Gu, X.F. Wang, N. Eisenhauer, T.J. Yang, J. Ma, Q.R. Shen, Y.C. Xu, and A. Jousset. 2016. Probiotic diversity enhances rhizosphere microbiome function and plant disease suppression. American Society for Microbiology, 7: e01790-16.

Jansa J, Vosátka M. 2000. In vitro and post vitro inoculation of micropropagated Rhododendrons with ericoid mycorrhizal fungi. Applied Soil Ecology, 15: 125 – 136.

Jin, H., P.E. Pfeffer, D.D. Douds, E. Piotrowski, P.J. Lammers, and Y. ShacharHill. 2005. The uptake, metabolism, transport, and transfer of nitrogen in an arbuscular mycorrhizal symbiosis. New Phytologist, 168: 687–696.

JMP®, Version <*15*>. SAS Institute Inc., Cary, NC, 1989–2022.

Johnson N.C., D.L. Rowland, L.Corkidi, L.M. Egerton-Warburton, and E.B. Allen. 2003. Nitrogen enrichment alters mycorrhizal allocation at five mesic to semiarid grasslands. Ecology, 84: 1895-1908.

Johnson NC, Wilson GWT, Wilson JA, Miller RM, Bowker MA. 2015. Mycorrhizal phenotypes and the law of the minimum. New Phytologist, 205: 1473– 1484.

Jongen, M., B. Albadran, W. Beyschlag, and S. Unger. 2022. Can arbuscular mycorrhizal fungi mitigate drought stress in annual pasture legumes? Plant and Soil, 472: 295-310.

Klironomos J.N. 2003. Variation in plant response to native and exotic arbuscular mycorrhizal fungi. Ecology, 84: 2292-2301.

Knight TM, J.A. Steets, J.C. Vamosi, S.J. Mazer, M. Burd, et al. 2005. Pollen limitation of plant reproduction: pattern and process. Annual Review of Ecology, Evolution, and Systematics, 36: 467–97.

Koide, R.T. 1991. Nutrient supply, nutrient demand and plant response to mycorrhizal infection. New Phytologist, 117: 365-386.

Koide R.T., Landherr, L.L., Besmer, Y.L., Detweiler, J.M. & Holcomb, E.J. 1999. Strate gies for mycorrhizal inoculation of six annual bedding plant species. HortScience, 34: 1217-1220.

Kovaleski, A.P., Williamson, J.G., Olmstead, J.W., and Darnell, R.L. 2015. Inflorescence Bud Initiation, Development, and Bloom in Two Southern Highbush Blueberry Cultivars. American Society for Horticultural Science, 140: 38-44.

Krishnamoorthy, R., K. Kim, C. Kim, and T. Sa. 2014. Changes of arbuscular mycorrhizal traits and community structure with respect to soil salinity in a coastal reclamation land. Soil Biology and Biochemistry, 72: 1-10.

Leake, J.R. and Read, D.J. 1990. Proteinase Activity in Mycorrhizal Fungi. New Phytologist, 115: 243-250.

Liu, S., H. Guo, J. Xu, Z. Song, S. Song, J. Tang, and X. Chen. 2018. Arbuscular mycorrhizal fungi differ in affecting the flowering of a host plant under two soil phosphorus conditions. Journal of Plant Ecology, 11: 623-631.

Maine, University of. Analytical Lab and Maine Soil Testing Service. Orono, ME.

Marschner, H. and Dell, B. Nutrient uptake in mycorrhizal symbiosis. 1994. Plant Soil, 159: 89–102.

Middleton, E. L., S. Richardson, L. Koziol, C. E. Palmer, Z. Yermakov, J. A. Henning, P. A. Schultz, and J. D. Bever. 2015. Locally adapted arbuscular mycorrhizal fungi improve vigor and resistance to herbivory of native prairie plant species. Ecosphere, 6: 276.

Nasholm, T., Hogberg, P., Franklin, O., Metcalfe, D., Keel, SG., Campbell, C., Hurry, V., Linder, S., and Hogberg, MN. 2013. Are ectomycorrhizal fungi alleviating or aggravating nitrogen limitation of tree growth in boreal forests? New Phytologist, 198: 214-221.

Neuhauser, C. and J.E. Fargione. 2004. A mutualism–parasitism continuum model and its application to plant–mycorrhizae interactions. Ecological Modelling, 177: 337-352.

NEWA. 1996-2022. NYS IPM Program, Cornell University. Version 1:0:1.

Pellegrino, E. and Bedini, S. 2014. Enhancing ecosystem services in sustainable agriculture: Biofertilization and biofortification of chickpea (*Cicer arietinum L.*) by arbuscular mycorrhizal fungi. Soil Biology & Biochemistry, 68: 429-439.

Peterson, L.A., E.J. Stang, and M.N. Dana. 1988. Blueberry response to NH4-N and NO3-N. Journal of the American Society for Horticultural Science, 113: 9-12.

Poorter, H., Bühler, J., van Dusschoten, D., Climent, J., and Postma, J. A. 2012a. Pot size matters: a meta-analysis of the effects of rooting volume on plant growth. Functional Plant Biology, 39: 839–850.

Read, D. J. 1996. The structure and function of the ericoid mycorrhizal root. Annals of Botany, 77: 365–374.

Reynolds HL, Hartley AE, Vogelsang KM, Bever JD, Schultz PA. 2005. Arbuscular mycorrhizal fungi do not enhance nitrogen acquisition and growth of old-field perennials under low nitrogen supply in glasshouse culture. New Phytologist, 167: 869–80.

Roveda-Hoyos, G., J.F. Venegas-Gomez, L.P. Moreno-Fonseca, S. Magnitskiy, and M. Ramirez-Gomez. 2022. Effect of inoculation with *Acaulospora* and *Glomus* on the growth and nutrition of blueberries (*Vaccinium corymbosum*) with different fertilization levels. Revista Colombiana de Ciencias Horticulas, 16: 1-12.

Scagel, C.F. 2005. Inoculation with ericoid mycorrhizal fungi alters fertilizer use of highbush blueberry cultivars. HortScience, 40: 786-794.

Scagel, C.F. and W.Q. Yang. 2005. Cultural variation and mycorrhizal status of blueberry plants in NW Oregon commercial production fields. International Journal of Fruit Science, 5: 85-11.

Shen S, Tu S-I, Kemper WD. 1997. Equilibrium and kinetic study of ammonium adsorption and fixation in sodium treated vermiculite. Soil Science Society of America Journal, 61: 1611–1618.

Smith, S. E., and D. J. Read. 2008. Mycorrhizal symbiosis, 3rd ed. Academic Press, San Diego, CA, USA.

Smith SE, Smith FA. 2011. Roles of arbuscular mycorrhizas in plant nutrition and growth: new paradigms from cellular to ecosystem scales. Annual Review of Plant Biology, 62: 227–250.

Taheri, W.I., and Bever, J.D. 2011. Adaptation of *Liquidambar styraciflua* to coal tailings is mediated by arbuscular mycorrhizal fungi. Applied Soil Ecology, 48: 251-255.

Thompson. J.N. 1988. Variation in interspecific interactions. Annual Review of Ecology and Systematics, 19: 65-87.

van der Heijden, M.G.A., Martin, F.M., Selosse, M.-A. and Sanders, I.R. 2015. Mycorrhizal ecology and evolution: the past, the present, and the future. New Phytologist, 205: 1406-1423.

Van der Heijden, M.G.A., N. Dombrowski, and K. Schlaeppi. 2017. Continuum of root-

fungal symbioses for plant nutrition. PNAS, 114: 11574-11576.

Van Geel, M., Jacquemyn, H., Peeters, G., van Acker, K., Honnay, O. and Ceulemans, T. 2020. Diversity and community structure of ericoid mycorrhizal fungi in European bogs and heathlands across a gradient of nitrogen deposition. New Phytologist, 228: 1640-1651.

Wang, B. and Qiu, YL. 2006. Phylogenetic Distribution and Evolution of Mycorrhizas in Land Plants. Mycorrhiza, 16: 299-363.

Ważny, R., Jędrzejczyk, R.J., Rozpądek, P. et al. 2022. Biotization of highbush blueberry with ericoid mycorrhizal and endophytic fungi improves plant growth and vitality. Applied Microbiology and Biotechnology, 106: 4775–4786.

Wei CZ, Yu Q, Bai E, Lu XT, Li Q, Xia JY, Kardol P, Liang WJ, Wang ZW, Han XG. 2013. Nitrogen deposition weakens plant–microbe interactions in grassland ecosystems. Global Change Biology, 19: 3688– 3697.

Wright, DP, Read, DJ., and Scholes, JD.1998. Mycorrhizal Sink Strength Influences Whole Plant Carbon Balance of *Trifolium repens L.* Plant, Cell and Environment 21: 881-891.

Wu, Q.S., R.W. Xia, Y.N. Zou. 2008. Improved soil structure and citrus growth after inoculation with three arbuscular mycorrhizal fungi under drought stress. European Journal of Soil Biology, 44: 122-128.

Xu Xinxiang, Du Xin, Wang Fen, Sha Jianchuan, Chen Qian, Tian Ge, Zhu Zhanling, Ge Shunfeng, Jiang Yuanmao. 2020. Effects of Potassium Levels on Plant Growth, Accumulation and Distribution of Carbon, and Nitrate Metabolism in Apple Dwarf Rootstock Seedlings. Frontiers in Plant Science, 11.

Zeng, L., L. JianFu, L. JianFu, and W. MingYuan. 2014. Effects of arbuscular mycorrhizal (AM) fungi on citrus quality under nature conditions. Southwest China Journal of Agricultural Science, 27: 2101–2105.

Zhu, X. C., F.B. Song, S.Q. Liu, and F.L. Liu. 2016. Arbuscular mycorrhiza improve growth, nitrogen uptake, and nitrogen use efficiency in wheat grown under elevated CO2. Mycorrhiza, 26: 133–140.

Zhang, C., Yin, L., and Dai, S. 2009. Diversity of root-associated fungal endophytes in *Rhododendron fortunei* in subtropical forests of China. Mycorrhiza, 19: 417–423.

Zinati, G. M., J. Dighton, and A. Both. 2011. Fertilizer, Irrigation, and Natural Ericaceous Root and Soil Inoculum (NERS): Effects on Container-grown Ericaceous Nursery Crop Biomass, Tissue Nutrient Concentration, and Leachate Nutrient

Quality, HortScience, 46: 799-807.