Getting Back to the Roots: AMF Colonization in Organically and Conventionally Grown Strawberry Rootstocks

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ABSTRACT

Arbuscular mycorrhizal fungi (AMF) helps plants absorb water and nutrients, possibly improving the quality of the plant. Organic farming methods generally encourage the growth of AMF better than conventional farming methods. To determine which specific agriculture practice affects the amount of AMF on strawberry rootstocks the most, I analyzed the effects of conventional and organic farming methods on initial AMF on rootstocks as well as the effect of individual farmers management methods' on AMF and fruit quality. I obtained rootstocks from three conventional nurseries in Northern California and counted a sample size of 20 from each nursery for initial AMF colonization. In the treatments, the initial AMF count significantly differed between the three different nurseries. Then, from the previous year's research that obtained data from four farms located in California's Central Coast that planted strawberries organically and conventionally, I analyzed AMF and fruit quality aspects and compared them with each other. When fruits were ripe and ready for harvest, the berries were collected to measure the weight and percent brix, or sugar content, and the roots were analyzed for AMF colonization. AMF colonization correlated with greater weight and higher percent brix. AMF, berry weight, and percent brix varied more between farm sites rather than organic and conventional fields. AMF, and consequently better fruit quality, depends more on which specific farming practices are used rather than whether conventional or organic practices are adopted.

KEYWORDS

Taste and ecology, mutualistic symbiont, agriculture practices, environmental health, nurseries and farmers

INTRODUCTION

Our current agricultural system causes many environmental problems, often using farming practices that trade-off between long-term maintenance of ecological process and short-term agricultural production (Foley et al. 2005, Raudsepp-Hearne et al. 2010). In the United States, farming practices fall under two main types: conventional and organic methods with organic being the most widely practiced and studied alternative to agriculture (Willer, H, Kicher 2011). Conventional farming relies heavily on synthetic chemical fertilizers, which not only cost billions of dollars per year but also contaminates water sources and habitats (Pimentel et al. 2005a). As opposed to conventional, the benefits of organic agriculture is that it aims to support plant nutrition, conserve water, and maintain soil health (Pimentel et al. 2005b). Yet many criticize organic farming methods for its failure in meeting the yields of conventional methods (de Ponti et al. 2012). Similarly, there is a presumption that organic systems are far better than conventional systems for its effect on the environment. High premiums of organic produce have incentivized farmers so that organic fields look identical to conventional large-scale monocultures which do little to sustain ecosystem health and biodiversity (Guthman 2004, Kremen et al. 2012). With the wide impact that agriculture has on the environment and society, finding improvements to both these methods can address many problems, including reduced biodiversity, eutrophication of surface waters, accumulation of pesticides, bacterial contamination, and low yields (Rembiałkowska 2007, Verbruggen et al. 2010). Thus, practices that promote natural mutualistic symbionts to improve the growth and health of plants is recognized as a promising method to reduce the effects of harmful farming practices (Hart and Trevors 2005).

Arbuscular mycorrhizal fungi (AMF) is one of the most ubiquitous soil microorganisms that has been shown to have a beneficial effect on crop productivity. AMF form mutualistic relationships with the roots of more than 80% of land plants (Wilson et al. 2009). AMF colonizes the roots of plants and delivers nutrients (e.g. nitrogen, phosphorus, and water) in return for carbohydrates (Douds and Millner 1999). AMF also helps support stronger, healthier, and higher-yielding plants by increasing nutrient uptake and reducing soil pathogens (Stewart et al. 2005). AMF also acts as a bio-protectant against soil pathogens and can contribute to the protection of host plants against pathogens by priming plant defense responses, causing microbial changes in the rhizosphere, and changing the root system, increasing the capacity for nutrient acquisition, and competing for the same ecological niche as fungal pathogens (Azcón-Aguilar et al. 2002, Board 2011). Based on its beneficial qualities, practices that aim to increase AMF are a useful alternative to fertilizers and fungicides. Therefore, nurseries and farmers can study and incorporate AMF more to provide robust rootstocks and maintain healthy plants that rely less on synthetic fertilizers or fungicides.

AMF promotes soil and plant health by forming symbiotic relationships with the roots of many plant species (Van Der Heijden et al. 2008). Because AMF helps absorb water and nutrients, it increases yields and positively affects fruit firmness, taste, maturity, and weight (Nicolás et al. 2014). Despite the known benefits, there is limited research on the effect of AMF on crop productivity in agricultural systems. In strawberry (*Fragaria ananassa*) production, rootstocks, rather than seeds, are used for planting as a result of their higher disease tolerance, quicker growth, increased yield, and better fruit quality (Lee Jung Myung 1994). Strawberry farmers obtain rootstocks from several nurseries to increase the chances of having successful crop (Guzman, *pers. comm.*). Because these rootstocks can vary in beneficial soil microbial properties before being planted by farmers, examining the initial AMF colonization on strawberry rootstocks can provide critical information for nurseries and farmers.

Strawberry management practices of both nurseries and farmers may have an impact on the amount of AMF colonization. More research is attempting to determine whether specific farm management practices affect mycorrhizal functioning (Barber et al. 2013). Conventional and organic farming can severely depress AMF colonization through use of many fertilizers and pesticides which limits biological activity and diversity below and above ground (Mäder et al. 2002, Oehl et al. 2004a, Reganold et al. 2010). Sustainable farming practices, on the other hand, can lead to an increase in overall microbial diversity and biomass, specifically AMF abundance (Oehl et al. 2004a). Nurseries provide rootstocks to both conventional and organic farmers. Therefore, evaluating the initial condition of strawberry rootstocks from nurseries is important to farmers as it will provide information as to where to buy rootstocks containing the highest mutually symbiotic AMF colonization.

This study determines if AMF presence, and subsequently fruit quality, is influenced by conventional, organic, or individual nursery and farm management practices. I answer the following questions: (a) Do management practices from the nursery and farm cause a difference in the presence of AMF colonization in strawberry roots? (b) Do conventional farming practices result in different initial AMF colonization in rootstocks from conventional nurseries? (c) Is there a difference in AMF colonization from conventionally and organically grown strawberries? If so, does it affect fruit quality (berry weight and percent brix)? And (d) Is there a difference in AMF colonization in strawberries grown from different farmers? If so, does it affect fruit quality?

I hypothesize that the initial AMF count of rootstocks from various conventional nurseries will not be significantly different from each other since they will all be in relatively low levels. I expect that organically grown rootstocks will have a higher proportion of AMF colonization, heavier berries, and higher percent brix than conventionally grown rootstocks. I also hypothesize that individual farmers' growing methods will have a greater impact on the amount of AMF, berry weight, and percent brix. Based on these results, I can determine if management methods or individual farmers' growing methods have a stronger impact on the amount of AMF colonization, percent brix, and berry weight. These findings can also help nurseries and farmers improve the quality of their rootstocks and crops by practicing farm management methods that increase AMF colonization and yield sweeter and bigger crops.

METHODS

Surveys

Nurseries

To determine the variety of strawberry for the focus of this study, I collected survey responses from nurseries through email. There were a total of twenty questions with questions such as how they grow their strawberries and whether they can provide rootstocks (see Appendix A for the survey). This information was used to determine what variety of strawberry to use and the specifics of the farming practices of nurseries. From the responses, I determined that the Albion variety would be the strawberry variety to focus on because the nurseries that responded all provided Albion. All the responses came from conventional nurseries. I received rootstocks from conventional nurseries, 68 from Nursery 1, over 200 from Nursery 2, and 100 from Nursery 3.

Study Site

Nurseries

I collected Albion strawberry rootstocks from three strawberry nurseries located in Northern California. All nurseries practiced conventional farming methods. I chose the Albion variety since all three nurseries carried this variety. Nurseries within a couple hours of driving distance from Berkeley were chosen to represent a sample of Bay area nurseries. The three nurseries are designated as Nursery 1 (36.91° N, 121.77° W), Nursery 2 (40.59° N, 122.30° W), and Nursery 3 (40.13° N, 122.27° W) for anonymity.

Farms

I analyzed data on AMF colonization and fruit quality from a research done the previous year by Aidee Guzman at the UC Berkeley Kremen lab from four farms, which will be referenced as Farm A, Farm B, Farm C, and Farm D. These four farms are located in California's Central Coast as well to sample Bay area farms and were chosen from a larger ongoing study on 30 farms in Dr. Claire Kremen's UC Berkeley lab. Guzman selected the four farms because they grew strawberries in two fields: one organic and the other conventional. Coordinates are respectively (-121.62° N, 36.77° W), (-121.55° N, 36.62° W), (-121.74° N, 36.89° W), (-121.69° N, 36.81° W). Guzman recorded observations of farming practices for each of the four farms.

Selecting and Labeling Rootstocks from Nurseries

To determine which rootstocks I would analyze, I randomly selected, labeled, and organized the roots into nursery groups. From the samples that were received from the three nurseries, I laid out the rootstocks and subsampled them for the study. The roots were placed in small paper bags labeled with the initials of the nursery and the root number between 1 and 68. Using a random number generator that selected numbers between 1 and 68 (68 since it was the lowest amount sent from the nurseries), I selected the first 20 randomized numbers because I would be using 20 rootstocks from each nursery as my sampling group. 20 rootstocks from each of the three nurseries formed a total of 60 randomly chosen rootstocks. The cassettes that I used to place the roots in were labeled with the nursery initials, group name, and number.

Selecting and Labeling Berries and Roots from Farms

To determine the effects of individual management practices on AMF and fruit quality, Guzman went to the four farms and selected the strawberry plants. She randomly selected three points inside a cluster of four plants within a plot that followed three criteria to obtain the berries and roots (Figure 1). The plot followed three criteria: at least 20 meters from the edge of the field, at least 10 meters from the road, and surrounded by 8-10 rows of healthy plants. She selected plants at least 20 meters in from the edge and at least 10 meters from the road to reduce confounding variables from external sources. To optimize selecting plants that would survive and be healthy for data collection, Guzman chose plants that were surrounded by other healthy plants.

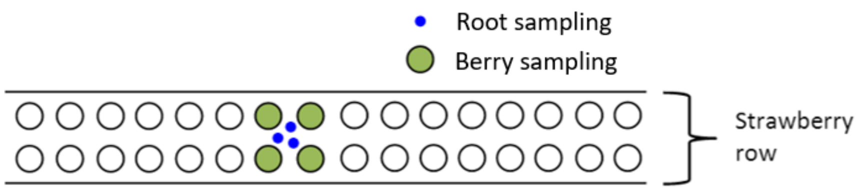

Figure 1. Berry and root selection. In a plot of strawberries that are at least 20 meters from the edge of the field, 10 meters from the road, and surrounded by 8-10 rows of healthy plants, a cluster of four plants were randomly selected for berry collection and three points in the cluster were selected for root collection.

Farm A, Farm B, Farm C, and Farm D were labeled with abbreviated initials of the farm names. If the berries and roots came from the organic field of the farm, they were labeled with 'A'. If they came from the conventional field, they was labeled with 'B'. The sampling time, either during the spring, summer, or fall, was also included in the label. If the berries and roots were sampled during spring than it was labeled with T1, if it was sampled during the summer than T2, and if it was sampled during the fall it was labeled with T3. Finally, the plant number was also recorded.

Measuring Strawberry Percent brix and Weight

To measure the strawberry percent brix, or percent brix, Guzman collected ripe berries from the sampled plants. They were refrigerated for 72 hours in paper bags labeled with their site and plant number. After 72 hours, she weighed the berries and recorded them according to their label. To measure the berry percent brix, Guzman crushed the berries and poured the juices on a refractometer. She recorded the percent brix according to the label. After every five berries, Guzman recalibrated the refractometer with distilled water. This procedure was repeated until the percent brix and weight of all the berries were measured.

Rootstock Preparation

To prepare the rootstocks for counting AMF colonization, I followed a modified protocol from a previous research (Koske and Gemma 1989, McGonigle et al. 1990). I washed the roots to remove soil and stored them in 25% ethanol until I was ready to stain them. Before staining, I drained the solution by rinsing the roots in water at least three times. Then I cut the roots into one centimeter fragmented subsamples and placed them into labeled histology cassettes. After boiling an appropriate volume of 3% KOH (3g/100ml or 30g/L) at 102°C, I added the histology cassettes to the boiling solution. I kept the cassettes with roots in the boiled KOH solution for thirteen minutes to further clean the roots and stirred three times during the period. Afterwards, I poured the KOH solution out. I rinsed the cassettes in water several times again until the water turned clear. Then, I soaked the cassettes with roots in 2% HCl for 30 minutes to stop the reaction and acidify the roots so the dye could be better absorbed. I boiled Trypan Blue solution at 110°C and added the histology cassettes. Once the solution reached 110°C again, I moved the beaker to a cold hotplate and set the cassettes in the hot solution for 30 minutes. I stirred the mixture every ten minutes. After rinsing the roots thoroughly under the cold tap to remove excess Trypan Blue, I placed the roots in the destaining solution. Again, I rinsed the roots thoroughly under the cold tap again to remove excess Trypan Blue. I stored the roots in 1:1 glycerol: water solution in a 1000 ml beaker in the fridge and let the roots sit in the refrigerator for two days before examining them. I conducted this procedure for both nursery and farm sources rootstocks.

Counting AMF Colonization

To count the AMF colonization, I mounted the rootstocks on slides. The procedure required mounting stained rootstocks in glycerin on microscope slides and covering with a cover slip. Singular pieces of roots were aligned in rows of five along the slide. I was careful not to remove the gel-like substance that surrounded the roots as that contains the AMF. The rootstocks from the previous year's research were already mounted on slides using the same method stated above and were ready to be counted for AMF colonization.

To count the AMF, I examined the roots by going up and down the slide from the top left corner of the slide towards the bottom right corner of the slide with each visible and countable root strand counting as one intersection, with 100 intersections made. If the root strand was too dark of a blue or brown, meaning the dye did not absorb well or the root was too thick, it did not count as a pass and the count was continued to the next countable strand. I used two clickers, one to tally if there was no AMF present, which was recorded as 0, and the other if there was AMF present, which was recorded as 1. If the root contained arbuscles, hyphae, or vesicles, it was counted as AMF being present.

Data Analysis

ANOVA and Kruskal-Wallis for Initial AMF Colonization

For the initial AMF data I collected from the three conventional nurseries, I used R commander in R to determine differences in AMF colonization from rootstocks in group T1 that came from different conventional nurseries. I used ANOVA to determine if there were significant differences in AMF proportion among nurseries followed by a Tukey HSD. I also used a Kruskal-Wallis test to determine the differences if the data was not symmetric. I also examined numerical summaries, that included the mean, median, standard deviation, and interquartile range, for AMF proportion which was the amount of AMF present divided by the total of AMF present and no AMF present.

The ANOVA and Kruskal-Wallis test compared the different pairings of nurseries rootstocks and its AMF presence. The null hypothesis is that there is no significant difference of

AMF colonization between the nursery pairings while the alternative hypothesis states that there is a significant difference between the two groups. This helped determine whether conventional farming methods by individual nurseries have an effect on the amount of initial AMF colonization in rootstocks. I used an alpha value of 0.05 and visualized my results with a boxplot.

ANOVA and Multilinear Regression Model for Comparing Conventional and Organic Roots' AMF, Percent Brix, and Berry Weight

To determine differences between the four farms' conventional fields with the farms' organic fields, I used R commander in R. I used ANOVA with the Tukey HSD to determine the effect of the conventional or organic farm management practice on berry weight and percent brix. I also used a Kruskal-Wallis test in case the results showed non-normal and unequal variances. I used multilinear regression models to determine the effect of conventional or organic AMF's effect on berry weight and percent brix. Numerical summaries that included the minimum, median, maximum, standard deviation, and interquartile range were also examined. I also coded boxplots of the farms' roots' AMF colonization in R studio to visualize the contrast and similarities between the organic and conventional roots. I created scatter plots with regression lines to see the correlation between AMF and percent brix as well as AMF and berry weight.

ANOVA and Multilinear Regression Model for Comparing Different Farms' AMF, Percent Brix, and Berry Weight

To compare the data between the different farms, I did similar methods of analysis done to compare the data between organic and conventional fields. I used ANOVA with Tukey HSD and Kruskall-Wallis to compare different pairings of the farm sites' AMF proportion, berry weight, and brix with each other. I also used multilinear regression models and linear regression models to find the correlation between the effect that the sites' AMF had on berry weight and percent brix. R commander and R studio were used to create numerical summaries, boxplots, and scatter plots with regression lines to analyze the data.

RESULTS

The proportion of initial AMF varied between rootstock sources. Nursery 3 had the lowest proportion of AMF present on its rootstocks with 0.03, while Nursery 1 and Nursery 2 had 0.41 and 0.44 respectively. Nursery 3 rootstocks had a significantly lower amount of AMF compared to Nursery 1 and 2's rootstocks with p-values that were less than 0.001. The boxplots of nurseries rootstocks' AMF proportion in Figure 2 portray that Nursery 3 rootstocks have a lower amount of AMF than the other nurseries' rootstocks (Figure 2). Since the data is not normal and the variances are unequal, I used the Kruskal-Wallis test which determined that the median AMF proportions from the three nurseries were significantly different from each other ($p < 0.001$).

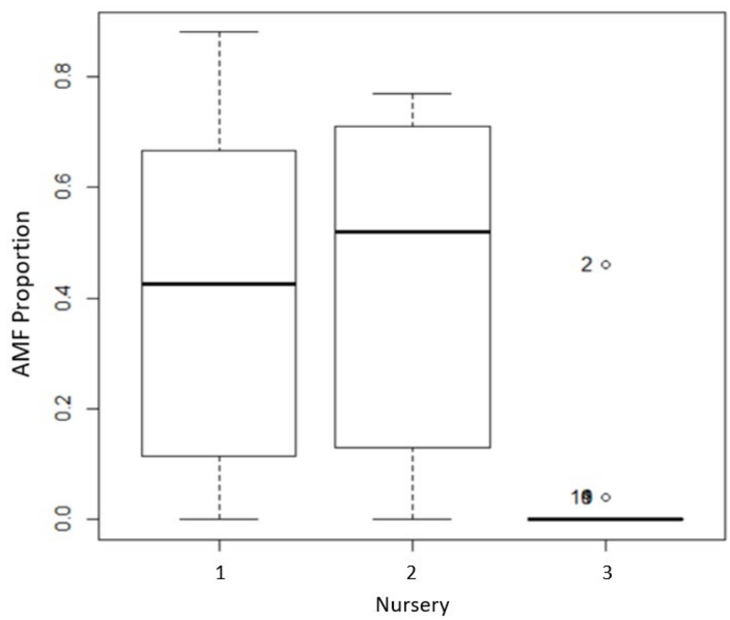

Figure 2. Boxplot of nursery AMF. Comparing boxplots of the proportion of AMF from the three different conventional nurseries, labeled 1, 2, and 3.

Comparing the organic and conventional fields, the difference in proportion of AMF in organic and conventional farms varied (Figure 3). Farm A and C had higher AMF proportion in organic fields while Farm B and D had higher AMF proportion in conventional fields. Only Farm B ($p=0.016$) and C ($p=0.021$) had significantly different values for AMF proportion between their organic and conventional sites. Overall, organic fields had an AMF colonization mean of 51.25 while conventional fields had a mean of 55.32. AMF proportion was not significantly different between organically and conventionally managed strawberry roots with a p-value of 0.920. Berry weight was significantly different between organic and conventional plots with a p-value of 0.018. Farm A (p=0.011) had significantly different berry weight between organic and conventional sites. Organic fields had a berry weight average of 17.06 g while conventional fields had an average of 22.16 g. The difference for percent brix was not significant between conventional and organic farms with a p-value of 0.113. None of the farms had significantly different percent brix between their organic and conventional sites. The average percent brix for organic fields was 9.71% and 8.88% for conventional fields. The Kruskal-Wallis test on AMF, berry weight, and percent brix resulted in p-values of 0.992, 0.030, and 0.139 respectively. Only berry weight had a significant difference for the Kruskal-Wallis test.

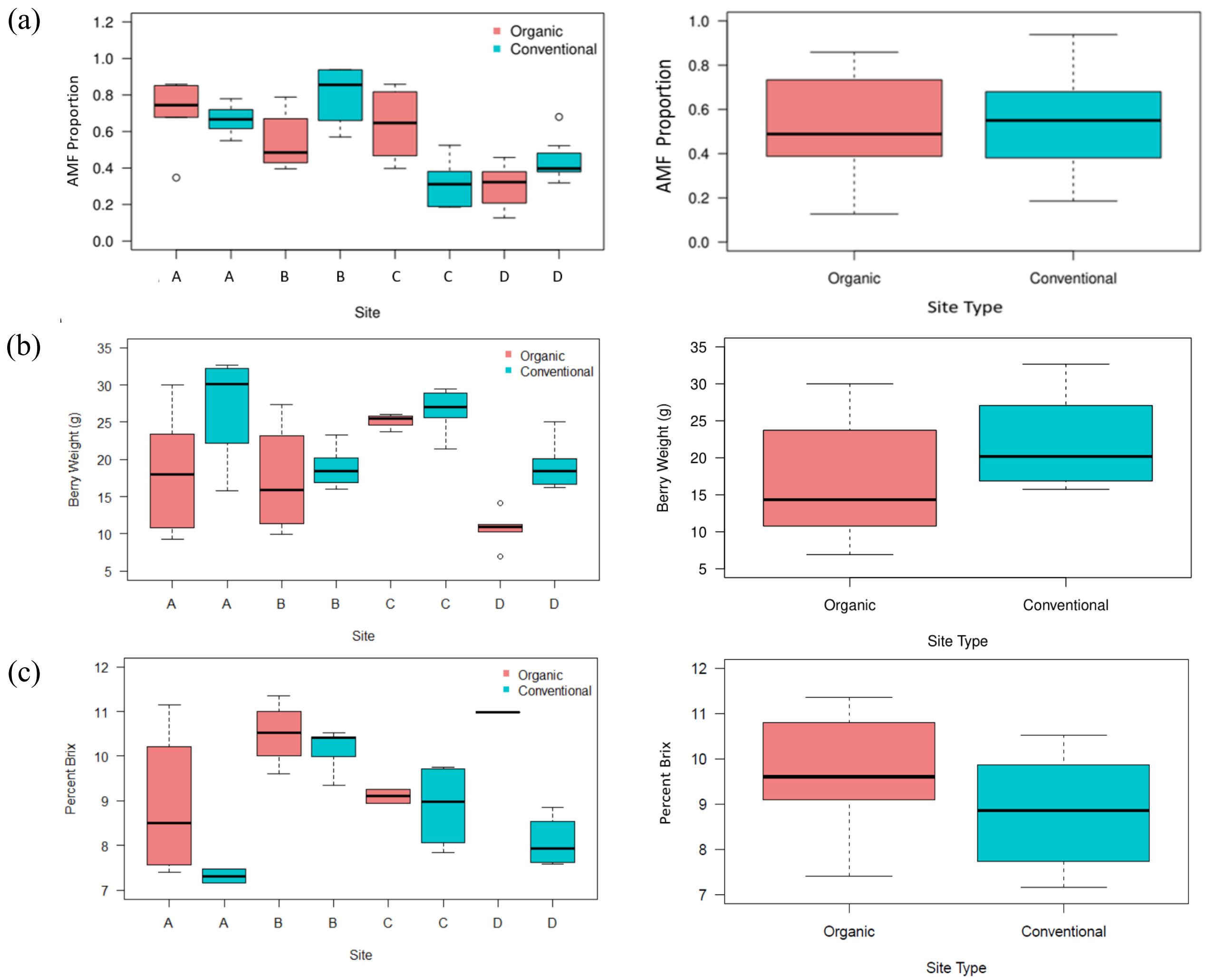

Figure 3. Differences in AMF colonization, berry weight, and percent brix based on site type. Box-and-whisker plots compare the differences in (a) AMF colonization between all the four farms' organic and conventional sites and the AMF colonization between the organic and conventional sites (b) the average berry weight between the four farms' organic and conventional sites and the average berry weight between the organic and conventional sites, and (c) the average percent brix between the four farms' organic and conventional sites and the average percent brix between the organic and conventional sites.

The effect of AMF on berry weight was not significant in either organic sites (p=0.672) or conventional sites (p=0.348). The effect of AMF on percent brix in organic sites (p=0.141) and conventional sites (p=0.149) was also not significant. These p-values are greater than 0.05 thus the relationship between AMF and berry weight and percent brix is not significant (Figure 4).

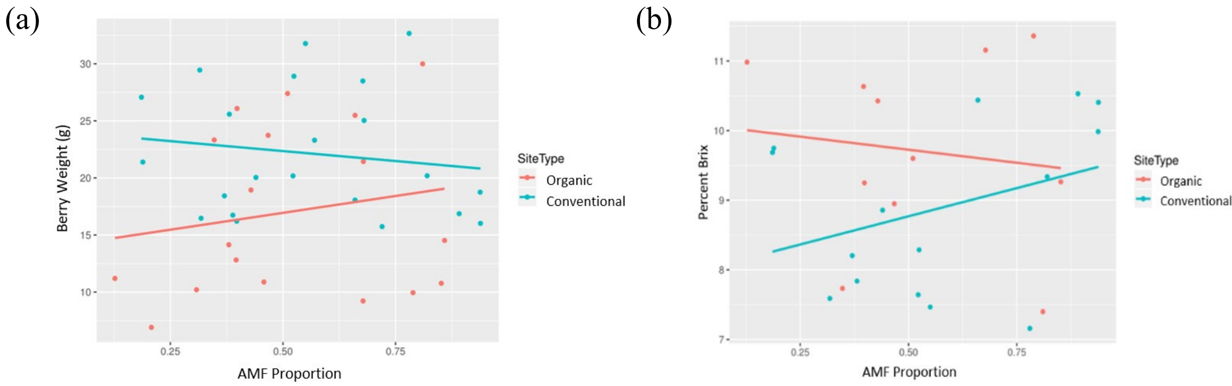

Figure 4. Differences in correlation between AMF colonization and berry weight and AMF colonization and percent brix based on site type. Scatter plots and regression lines show the weak correlation between AMF count to (a) berry weight and (b) percent brix.

When comparing the AMF between the four different farm sites, Farm A had the greatest amount of AMF present on their rootstocks with a proportion of 0.69 while Farm D had the least with a proportion of 0.38 (Figure 5). Farm B was slightly less than Farm A with 0.67 and Farm C was lower with 0.48 (Figure 5). Berry weight was greatest for Farm C (25.97 g and least for Farm D (15.54 g). Percent brix was highest for Farm B with 10.30% and the other farms had a similar percentage with Farm C, Farm D, and Farm A having percentages around 8.36%, 8.96%, and 8.67% respectively. Using the Tukey HSD, I saw that the differences in AMF proportion between Farm A – Farm C, Farm A – Farm D, and Farm B – Farm D were significantly different from each other with p-values of 0.03, <0.001, and <0.001 respectively. The other pairs, Farm A – Farm B, Farm B – Farm C, and Farm C – Farm D did not have AMF count significantly different from each other with p-values greater than 0.05. For berry weight, all farm pairs had insignificantly different berry weight except for Farm B – Farm C with p-value of 0.04 and Farm C – Farm D with p-value of 0.003. For percent brix, all pairs of farms had non-significantly different percent brix levels from each other except for Farm A – Farm B with a p-value of 0.01. Using the Kruskal-Wallis test, the difference between the AMF proportion from the four farms was significant ($p < 0.001$). The difference for berry weight for the four farms was significant with a p-value less than 0.001. For percent brix, the p-value was 0.02. The differences in AMF, berry weight, and percent brix vary between farm sites and show more specific relationships between the farms (Figure 5). Using the multilinear model, Farm A ($p<0.001$) and D ($p<0.001$) had significantly different AMF proportion than the rest with Farm B ($p=0.061$) close to being significantly different. For berry weight, Farm A ($p<0.001$) and D ($p=0.022$) also had significantly different values. Percent brix was significantly different for farms A ($p<0.001$) and B ($p=0.024$) and close for Farm D ($p=0.058$).

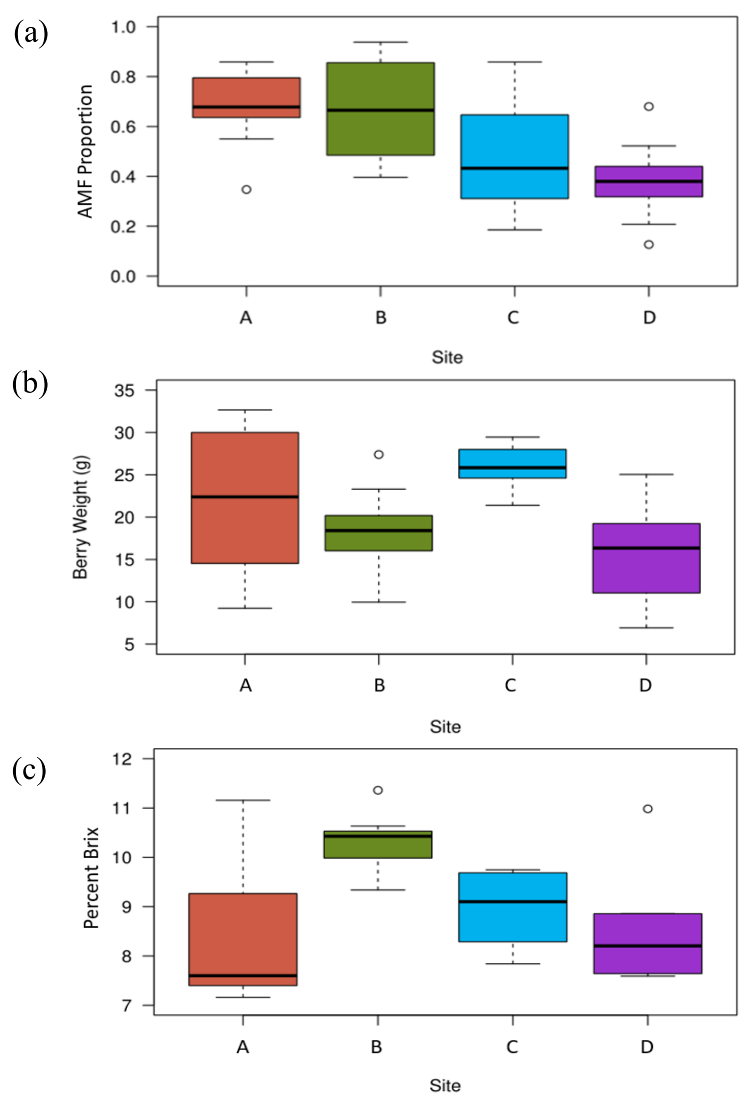

Figure 5. Differences between AMF colonization, berry weight, and percent brix based on site. Box-and-whisker plots compare the differences between (a) AMF colonization, (b) berry weight, and (c) percent brix between the four different farms.

When comparing AMF to berry weight and to brix levels for the farms, there is a stronger correlation than the relationship between the organic and conventional farm sites (Figure 6). Yet only Farm D for the effect of AMF on berry weight was significantly different from the other farms with a p-value of 0.002 (Figure 6). All of the farms had various slopes which show that AMF proportion did not have a significant effect on berry weight and percent brix.

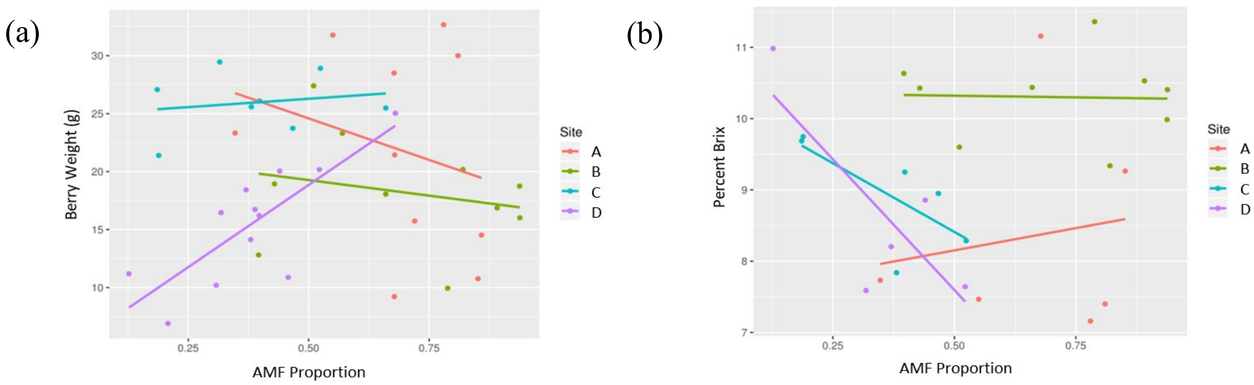

Figure 6. Differences in correlation between AMF colonization and berry weight and AMF colonization and percent brix based on site. Scatter plots and regression lines show the correlation between AMF count to (a) berry weight and (b) percent brix.

DISCUSSION

The levels of initial AMF colonization was significantly different between the conventional nurseries. For the strawberries grown in different farm plots, organically grown rootstocks had non-significantly smaller amount of AMF colonization compared to conventionally grown rootstocks. Berry weight was significantly different between organically and conventionally grown rootstocks with conventionally grown strawberries having a higher berry weight. There was not a significant difference for percent brix between organically and conventionally grown strawberries. In a comparison between farms, there were significant differences in AMF colonization, berry weight, and percent brix. These relationships reveal that although organic management practices may not have a significant effect on encouraging AMF growth than conventional management practices, individual farmers' specific management practices have a greater impact on the presence of AMF colonization in strawberry roots. This information can benefit individual farmers who would want to encourage the growth of natural symbiotic AMF on their crops.

Differences in AMF from Conventional Nurseries' Rootstocks

The varying amount of initial AMF colonization on the rootstocks from the three different conventional nurseries suggest that conventional farming methods do not always result in rootstocks with low AMF. As the boxplots were not normal and the variances were unequal, the p-values from the Kruskal-Wallis test were used. Nursery 3 grew rootstocks very low in AMF compared to rootstocks from Nursery 1 and Nursery 2. Certain AMF species are greatly impacted by soil management practices, including tilling (Jansa et al. 2002). I expected the conventional rootstocks to all have a similar low level of AMF because chemical fertilizers have harmful effects on AMF (Moeskops et al. 2010), thus the significant difference between certain nurseries was unexpected. This difference may be due to the different farming methods that each nursery used, suggesting that the methods of individual farmers or location may have a greater impact on the amount of AMF colonization on roots (van der Gast et al. 2011). Nursery 3 used herbicides to manage weeds and common sulfur as a soil amendment which was the main difference from the practices of other nurseries. While there is not much research on the effects of sulfur on AMF, herbicides may have a negative effect on AMF colonization by impairing the vitality of the host plant (Jansa et al. 2006).

Differences in AMF from Conventionally and Organically Grown Strawberries

The mean level of the AMF colonization in conventional sites was similar to the mean level of AMF colonization in organic sites, suggesting that conventional practices do not have a debilitating effect on AMF presence. This non-significant difference contrasted to my hypothesis that organically managed roots would have a significantly higher amount of AMF presence. The low sample size may have contributed to the results that contradict other studies' results that say organic plots have higher amount of AMF than conventional ones. For example, a study by Reganold et al. (2010) looked at strawberries from thirteen conventional and organic strawberry fields and found significant results in favor of organic practices benefitting AMF growth.

Figure 7. Side-to-side comparison of the organic and conventional plots in Farm A. The left side of the photo shows the organic plot and the right side shows the conventional plot.

Looking at each of the four farms differences between organic and conventional sites, variations in AMF could be explained by the farms' specific practices. Farm A had organic and conventional sites that looked identical (Figure 7). Aspects that help improve soil health, such as hedgerows, natural areas, and mixed crops, are lacking in both Farm A's conventional and organic sites (Kremen et al. 2012). Thus, the amount of AMF is not significantly different from each other. Farm B is a small-scale farm of 10 acres and surrounded by diverse vegetation. Farm B's high AMF count in conventional sites could be explained by the farmer overcompensating for the conventional method by putting more soil inputs to improve soil health in fear of losing their crop (Jansa et al. 2014). Farm C had very large berries from their sites. Fertilizer may have been added to the conventional site to grow larger berries which would decrease the dependence the crops have on AMF (Dodd and Dodd 2000). Finally, for Farm D, the strawberries were small and unhealthy from both sites which may have been due to disease or poor soil health which would contribute to low amounts of AMF (Jansa et al. 2014).

Differences in Berry Weight and Percent Brix from Conventionally and Organically Grown Strawberries

Berry weight was significantly higher in conventional fields than organic ones while percent brix was not significantly higher in organic fields than conventional ones. All four farms' organic sites had strawberries with lower berry weight than the conventional sites' strawberries. This suggests that organic practices may not always improve fruit quality. The weak correlation between the AMF proportion and berry weight for both organic and conventional sites as well as the contradicting slopes for the organic and conventional regression lines show that AMF does not greatly contribute to higher berry weight.

On the other hand, percent brix was lower in all four farms' conventional sites than organic sites. Overall though, the difference between organic and conventional sites' percent brix was not significant. This suggests that organic sites may not have a significant impact on percent brix. The weak correlation for the organic and conventional sites' effect of AMF on percent brix as well as the contradicting slopes for organic and conventional sites' regression lines show AMF did not greatly affect percent brix in either management types. Other studies have shown that inoculating strawberry plants with AMF resulted in higher glucose and sucrose content compared to berries not inoculated with AMF through supplying nutrients such as phosphorous (Surendran and Vani 2013, Bona et al. 2015). Yet Vázquez-Hernández et al. (2011) found that for papayas, AMF did not affect fruit percent brix. However, this research focused on papayas and the lower percent brix could have been because of dilution due to excess rain water a few days before harvest time. A greater factor on berry weight and percent brix could have been specific farming practices.

Differences in AMF from Strawberries Grown by Different Farmers

Farm A and Farm B contained roots with significantly higher amounts of AMF than Farm D and Farm A also had significantly higher AMF colonization than Farm C which suggests that the differences in Farm A and B to Farm C and D may show what influences AMF presence. There were also more significant differences in AMF between different sites than different site types which suggests that specific farming practices have a greater impact on AMF colonization. Individual farming methods that focus on using less chemical inputs, preventing soil erosion, improving soil matter, and increasing biodiversity have a significant impact on the amount of beneficial AMF on roots (Pimentel et al. 2005b). Farm A and B did not use cover crops, used soil root stimulators, put in a higher proportion of soil improvement input, and used pellet compost which parallels the concept in the research done by Pimentel et al (2005b). Farm D also had the least healthy seeming strawberry plants which may have been due to a crop disease and poorer soil health which would reduce AMF proportion (Jansa et al. 2014). Farm C also had the largest strawberries which may have been due to including fertilizers to enhance the growth which would decrease the dependence on AMF.

Differences in Berry Weight and Percent Brix from Strawberries Grown by Different Farmers

Strawberries from Farm C had significantly heavier berries than strawberries from Farm D and Farm B which suggests that Farm C's specific farming practices, although they may not support the growth of AMF, support the growth of larger berries. Farm C uses manure compost while the other farms do not, which may have contributed to heavier berries as manure compost improves soil properties and increases organic matter and nutrients (Wong 1999). Farm A and B, both of which had significantly higher levels of AMF colonization, had the second and third heaviest berry weight. Farm D, with the lowest amount of AMF colonization, also had the lightest berry weight. The correlation between AMF proportion and berry weight suggests that while the presence of AMF colonization may encourage the growth of heavier berries, specific farming practices may have a greater impact on berry weight. More knowledge in this area could be done to see what specific farming practices affect berry weight. A study by Al-Karaki and Al-Karaki (2000) on tomatos shows that the ability of AMF to increase the uptake of water and nutrients with low mobility for crops increases fruit size.

Farm B had the highest percent brix and a significantly higher brix level than Farm A. Farm C had the second highest percent brix levels. The main difference between Farm B and C to Farm A and D was that Farm B and C use soil input biocatalysts. There has not been many research that suggests the positive effects biocatalyst have on the percent brix of strawberries. Again, more research could help determine which specific farming management practices have a significant impact on percent brix in fruits. There were more significant differences in fruit quality between different farms than the conventional and organic comparison which reveals that management practices of individual farmers have a greater impact on fruit quality.

Limitations and Future Directions

The number of nurseries I sampled was very small relative to the number of nurseries in Northern California. The small sample size is not a good representative of all the conventional strawberry nurseries' rootstocks which may explain why the results contradict other research on the negative effect that conventional methods have on AMF. In Oehl et al's (2004b) research, only four plots were analyzed to determine the diversity of AMF yet it was long-term study that lasted for 22 years. Even with the small sample size, the duration of the study could have contributed to results that fit well with other observations in increased microbial presence in organically managed fields (Fliessbach and Mäder 2000). I also originally planned on sampling from organic nurseries to compare the initial AMF between organic and conventional farming methods but obtaining strawberry rootstocks from organic nurseries proved more difficult than obtaining rootstocks from conventional nurseries. The difficulties in obtaining organic rootstocks reveal the greater prevalence and perhaps issue of organic farmers being able to obtain organic rootstocks compared to conventional rootstocks.

Comparing the initial amount of AMF between conventional and organic rootstocks can provide further information on the effects of these farming methods on AMF colonization. A future study could exploring growing these roots in their original farming method and in the opposite farming method to see the impact that the duration of the farming method has on the presence of AMF. Testing if there is a significant difference in AMF colonization between the initial count and the count after strawberries are grown will bring further evidence for or against which farming practice has a greater impact on AMF presence. Soil content can be another variable that can be analyzed to see the effects a farmer's management has on the environment for AMF. Identifying the species of AMF would also be useful for farmers to know which methods encourages the growth of a diverse range of AMF because a diverse range brings out the full range of benefits of AMF to host plants (Klironomos 2003, Gosling et al. 2006, Johnson and Graham 2013).

Surveys of the nurseries and farms' management practices provided a limited view of the impact that farming management practices have on AMF and fruit quality. A more in-depth knowledge of the chemicals and fertilizers used on the rootstocks as well as on the organic and conventional sites would provide helpful information on the effects of specific farming practices. A long-term field study of several strawberry farms with various farming practices and an in-depth record of the different inputs and farming methods used in the conventional and organic sites could shed more light on the specific farming practices' effect on AMF colonization and fruit quality. This would provide more information to nurseries and farmers on what specific management practices they could adopt to improve their crops and field.

Conclusions

The possibility that individual farming methods have a significant influence on the amount of AMF on rootstocks can be useful information for farmers and nurseries. Determining the farming practice that best supports farm ecosystem and fruit health is more complex than comparing conventional and organic practices. Instead of supporting the underdeveloped belief that organic is better than conventional, this study supports the less implemented belief that individual sustainable strategies have a greater impact on improving ecosystem and crop health (Dodd and Dodd 2000). Because organic systems can be very similar to conventional ones through practicing monoculture and using a high input of purchased compost and soil amendments, there should not be a presumed dichotomy between organic and conventional farming practices (Kremen et al. 2012). There were few significant differences in AMF presence, berry weight, and percent brix between organic and conventional plots. The few significant differences may have been attributed to different farming practices. Thus, individual farming methods have a greater impact on AMF proportion and fruit quality. These results provide greater information for farmers to know how to produce crops that encourages the growth of symbiotic AMF, have better taste, and are bigger in size. By understanding the wide range of specific management practices and their effect on the two factors, taste and ecology, farmers can improve their crop quality as well as their farm health.

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APPENDIX A: Survey of Nurseries

1

Project title: Arbuscular Mycorrhizal Fungi in Strawberry Rootstocks

Participant Survey

Name of nursery: ______________________ City: ______________________

Size of Nursery: ______________________ Years rented: ______________________

Total acres owned: ______________________ Years managed land: ______________________

Total acres rented: ______________________

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1. **What variety of strawberries do you grow?**
   - ☐ Albion
   - ☐ Aromas
   - ☐ Keoki
   - ☐ Monterey
   - ☐ Portola
   - ☐ San Andreas
   - ☐ Seascape
   - ☐ Sweet Ann
   - ☐ Benicia
   - ☐ Camarosa
   - ☐ Camino Real
   - ☐ Chandler
   - ☐ Hood
   - ☐ Palomar
   - ☐ Florida
   - ☐ Fronteras
   - ☐ Stella
   - ☐ Festival
   - ☐ Petaluma
   - ☐ Cabrillo
   - ☐ Sensation
   - ☐ Sequoia
   - ☐ Honeoye
   - ☐ Allstar
   - ☐ Éclair
   - ☐ Ft. Laramie
   - ☐ Ozark
   - ☐ Quinault
   - ☐ Eversweet
   - ☐ Sweet Charlie
   - ☐ Tillamook
   - ☐ Valley Red
   - ☐ Ventana
   - ☐ Lucia
   - ☐ Scarlet
   - ☐ Ruby June
   - ☐ Jewel
   - ☐ Flavorfest
   - ☐ Radiance
   - ☐ Other (*please name*):__________
2. **How would you describe your management practice?**
   - ☐ Organic
   - ☐ Conventional
   - ☐ Both
   - ☐ Other: ______________________
3. **The typical rotation for my strawberry crop is:**
   - ☐ 2-year rotation (one year of current crop and one year of different crop)
   - ☐ 3-year rotation (one year of current crop and 2 years of different crops)
   - ☐ 4-year rotation (one year of current crop and 3 years of different crops)
   - ☐ 5(+)-year rotation (one year of current crop and 4(+) years of different crops)
4. **What type of cover crop do you use for your strawberry?**
   - ☐ Leguminous cover crops (including clovers, hairy vetch, field pea)
   - ☐ Fast-growing cover crops (including buckwheat, sorghum-Sudangrass, Japanese millet)
   - ☐ No cover crop

2

5. Which of the following weed management practices do you use?
- ☐ Spot spray or cultivate for weeds if weed is spotted in field
- ☐ Use mechanical methods (hilling, cultivation, rotary hoeing)
- ☐ Herbicide chemistries in rotational crops to avoid potential resistance
- ☐ Plant cover crops
- ☐ Tilled after harvest
- ☐ Spot spray herbicides before planting
- ☐ Clean equipment before use

6. Which of the following insect management practices do you use?
- ☐ Rotate classes of insecticides to avoid resistance
- ☐ Select an insecticide to enhance/preserve natural enemies
- ☐ Scout for insect pests at critical periods
- ☐ Manage crop to improve crop health to increase crop resilience

7. When do you use fungicides?
- ☐ Before planting on soil (drip irrigation, spray, etc.)
- ☐ On the rootstocks before planting
- ☐ After planting on soil/foliage (drip irrigation, spray, etc.)

8. When do you use pesticides?
- ☐ During early stages of insect and weed infestation
- ☐ Once pests have been identified in fields and as needed as infection progresses
- ☐ Until levels of the pest have reached or exceeded established thresholds
- ☐ Not used

9. Do you use any soil amendments?
- ☐ Sphagnum peat moss
- ☐ Humus
- ☐ Composted manure
- ☐ Mushroom compost
- ☐ Topsoil
- ☐ Lime
- ☐ Sulfur
- ☐ Gypsum
- ☐ Perlite
- ☐ Vermiculite
- ☐ Builder's sand
- ☐ None

10. Do you use AMF inoculum?
- ☐ Yes
- ☐ No

11. What type of fertilizer do you use?
- ☐ Synthetic
- ☐ Organic
- ☐ None
- ☐ Other: ____________________

12. How much water was used to grow this crop per week?
- ☐ 1 inch per acre
- ☐ 2 inches per acre
- ☐ 3+ inches per acre

3

13. What method was used to water the strawberry crop?
- ☐ Overhead watering
- ☐ Drip irrigation
- ☐ Soaker hose
- ☐ Other: ____________________

14. Do you have a target pH?
- ☐ 5.0-5.4
- ☐ 5.5-5.9
- ☐ 6.0-6.4
- ☐ 6.5-6.9

15. Do you add anything to the soil to maintain a certain pH?
- ☐ Phosphorous-and-potassium-rich fertilizer
- ☐ Aluminum sulfate
- ☐ Common sulfur
- ☐ Ferrous sulfate
- ☐ Compost
- ☐ Peat moss
- ☐ Manure
- ☐ Other: ____________________

16. How many farms do you supply strawberry rootstocks?
- ☐ 0-20
- ☐ 21-40
- ☐ 41-60
- ☐ 60+

17. Where are the farms you supply strawberry rootstocks located?
- ☐ Southern California
- ☐ Northern California
- ☐ Central California

18. How long have you been in business?
- ☐ 0-10 years
- ☐ 11-20 years
- ☐ 21-30 years
- ☐ 30+ years

19. Could you send at least 50 rootstocks for us to study?
- ☐ Yes
- ☐ No

20. When is the earliest date that you can send the rootstocks?
- ☐ Date (MM/DD/YY): ________________

Thank you for completing the survey!