Determining Colonization Efficacy of Commercial Mycorrhizae Products and *Fragaria* x *anannassa* 'Albion'.

A Senior Project

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by

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Introduction

The plant production industry is continually expected to produce high commodities for a demanding population that is growing at an even exponentially faster rate. To effectively meet such demands, crop protection has become the top priority and has necessitated the development of high-input management systems to minimize losses. These management systems are effective to certain degrees, but the resulting increase in production costs and newly resistant pest populations, as well as the concern from the public sector regarding the longevity of such practices, have spurred advances towards more sustainable methods for maintaining plant health.

Mycorrhizae fungi are a category of beneficial fungi capable of forming symbiosis with a host plant where bidirectional nutrient exchange is known to occur, generally resulting in enhanced plant vigor and health. The fungi, in return, utilize the host plant as a source for carbohydrates. The implications of incorporating this symbiosis into plant production could mean reducing the dependency for high cost input production systems, in virtue of this increased nutrient uptake and vigor.

Strawberries are commercially grown in California for the fresh market. Finding mycorrhizae capable of colonizing this cash crop would provide further knowledge as to which mycorrhizal species can be propagated for repeatable success and provide greater sustainable and cost effective methods for mass production. The goal of this experiment was to test the efficacy of colonization between two commercial mycorrhizal inoculants of varying species counts with *Fragaria* x *anannassa* 'Albion' when grown under greenhouse conditions.

Literature Review

Identifiable morphological features during for mycorrhizal symbiosis to initiate includes mycelium mats comprised of vast, branching hyphae found below ground within the host

rhizosphere. Root vicinity is imperative for spore germination to initiate and for early directional growth of hyphae towards a host, as the exudates from the host roots contain the various chemical compounds secreted into the rhizosphere and function as the attractant for the fungal directional growth. Studies *in vitro* using root organ culture have reported that higher concentrations of carbon from root exudates are directly related to both the increased branching and density of hyphae (Douds & Nagahashi, 2000).

The morphology of the mycelium benefits the host plant primarily through the enhanced uptake and transportation of soil nutrients by providing an extension into the nutrient pool beyond the plants pre-inoculated capacity. The mycelial network acts like an extension to the root system, capable of absorbing and transporting mobile nutrients (such as nitrogen) and the colloid-bound immobile nutrients (such as copper, zinc and phosphorous) from soil sites beyond the depletion zone of deficient roots. In comparison to a root, the smaller diameter of the hyphae strands further increases the surface area in contact with the soils, thereby increasing the nutrient uptake by host plants (Bücking, 2012). To ensure a higher probability of fungal colonization of the host, roots within the vicinity of spores are imperative to initiate germination. Research has generally observed the root exudate in particular acting as a regulator for hyphae morphogenesis (Giovannetti *et al*., 1993).

Materials and Methods

The experiment was conducted in a research greenhouse at the crops unit of California Polytechnic State University in San Luis Obispo, CA (35.3017° N, 120.6598° W), from November 2014- May 2015. Thirty-six Fresh strawberry crowns of *Fragaria* x *ananassa*

‘Albion’ were transplanted into one-gallon plastic nursery pots containing a soilless medium of 2:1 peat moss to perlite. The soil was moistened before the roots and crowns were planted, and watered until field capacity after transplanted.

The transplanted pots were then placed into the research greenhouse and onto a wire bench measuring 4 feet by 30 feet, totaling 120 sq-ft with 1' spacing between each pot. Temperature in the greenhouse were maintained between 55 degrees and 75 degrees. Supplemental fans were placed on the east and west side of the bench and scheduled to go on for 30 min every three to four hours.

Subjects were then treated with a liquid Fox Farm (6-4-4) starting fertilizer and watered when needed. After 21 days, the Fox Farm applications were substituted with Osmocote (15-9-12) 4-6m slow release fertilizer. 66 days later, additional applications of Jack’s Professional (20-10-20) soluble fertilizer was added as a calcium source applied once weekly for the duration of the experiment.

The 36 subjects were divided equally into three groups of 12, comprised of two treatment groups and one untreated control group. Inoculation for treatment groups one and two (T1 and T2) occurred on Dec. 24th. T1 received the multi-species product MycoApply, with 1mL containing 120 propagules/gram of *Glomus intradices, Glomus mosseae, Glomus aggregatum* and *Glomus etunicatum* and T2 received the single-species product MycoUp, with 1mL containing 70 propagules/gram of *Glomus iranicum.* Groups T1 and T2 were inoculated on Dec. 24th, then reinoculated on March 2nd with their respective treatments. The treatments were applied according to the instructed label.

Figure 1. Randomized block design and shoot growth differences between Jan.28 (left) and Feb. 11 (right).

Cross contamination due to irrigation runoff was addressed by elevating the pots above the wire bench using insulation foam blocks, as well as from barrier which prevented splashing spores from making contact with nearby subjects.

Data Collection:

The data sets were collected by utilizing destructive harvest techniques at the end of the experiment.

Staining

Three plants were randomly selected from each group to undergo microscopic analysis to determine if successful mycorrhizal colonization occurred and to what degree in virtue of calculating percentage of root colonization. Root clearing and chitin (fungal-wall) staining techniques were used to locate any present fungus-root interfaces (Comas *et al.,* 2011). The 9 selected were severed from their roots, which were then soaked in the following solutions: (1) 10% NaOH solution (2) ammonia/hydrogen peroxide solution and (3) 5% HCl, until enough cellular contents had cleared for the stele to become visible. 0.05% Trypan blue was used to stain any present fungal walls to reveal the fungal structures. Both dissecting and compound microscopes were utilized for root analysis

Figure 2. Staining preparation of selected T2 subjects undergoing root clearing

Shoot/Root weights

Of the 27 remaining subjects, shoots were severed and individually weighed for their fresh mass. Roots were then removed from the soil and weighed for their fresh mass (Figure 3)

Data was analyzed for normality and statistically significant differences were reviewed using ANOVA and Tukey HSD.

Figure 3. T3 control subject roots after severed from shoots.

Fruit Weight:

Strawberry fruits were harvested once between March 3rd and March 9th and weighed. Fruits per subject qualified for harvesting once the majority reached a red color and averages for each treatment was observed.

Results

Shoot and Root Weights:

Shoot and root weights were measured for each subject. T1 plants inoculated with the multi-species product averaged 61.21g shoot weight and 32.9 root weight, T2 plants inoculated

with the single-species product averaged 88.23g shoot weight and 42.7 root weight and non-inoculated control plants averaged 75.56 shoot weight and 20.8 root weight (Chart 1.) One-way ANOVA results indicated no significant differences (p=0.05) in both the shoot weights and root weights for all treatment groups (Tables 1 and 2).

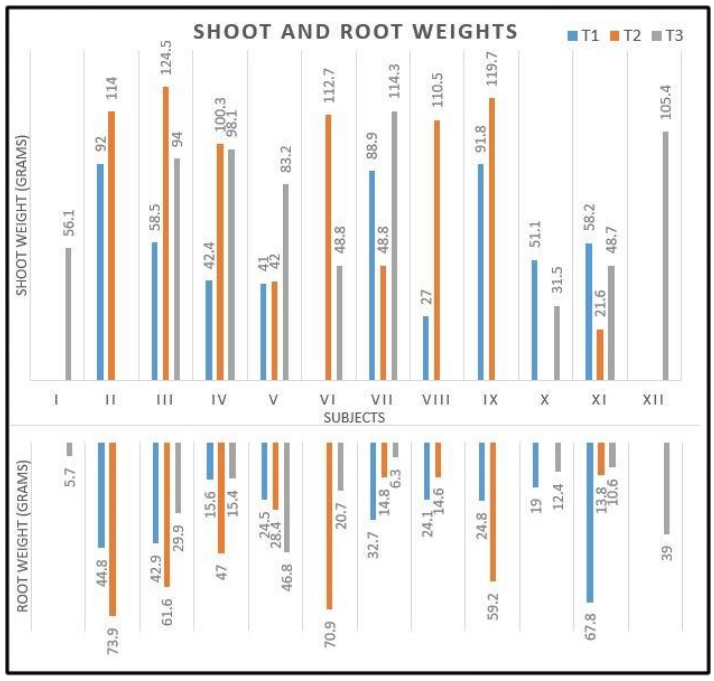

Chart 1. Shoot and root weight measurements for remaining subjects.

ANOVA						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	3501.0496 3	2	1750.5248 15	1.7478638 54	0.1955925 37	3.4028261 05
Within Groups	24036.537 78	24	1001.5224 07			
Total	27537.587 41	26				

Table 1. One-way ANOVA with replication of shoot weight.

Between Groups	2157.428 889	2	1078.714 444	2.901545 201	0.07437 026	3.402826 105
Within Groups	8922.537 778	24	371.7724 074			
Total	11079.96 667	26				

Table 2. One-way ANOVA with replication of root weight.

Fruit Weights:

The T3 uninoculated control group yielded the highest average fruit weight at 147g/treatment, while the T2 group treated with the single-species product yielded the lowest average fruit weight at 89.4g/treatment.

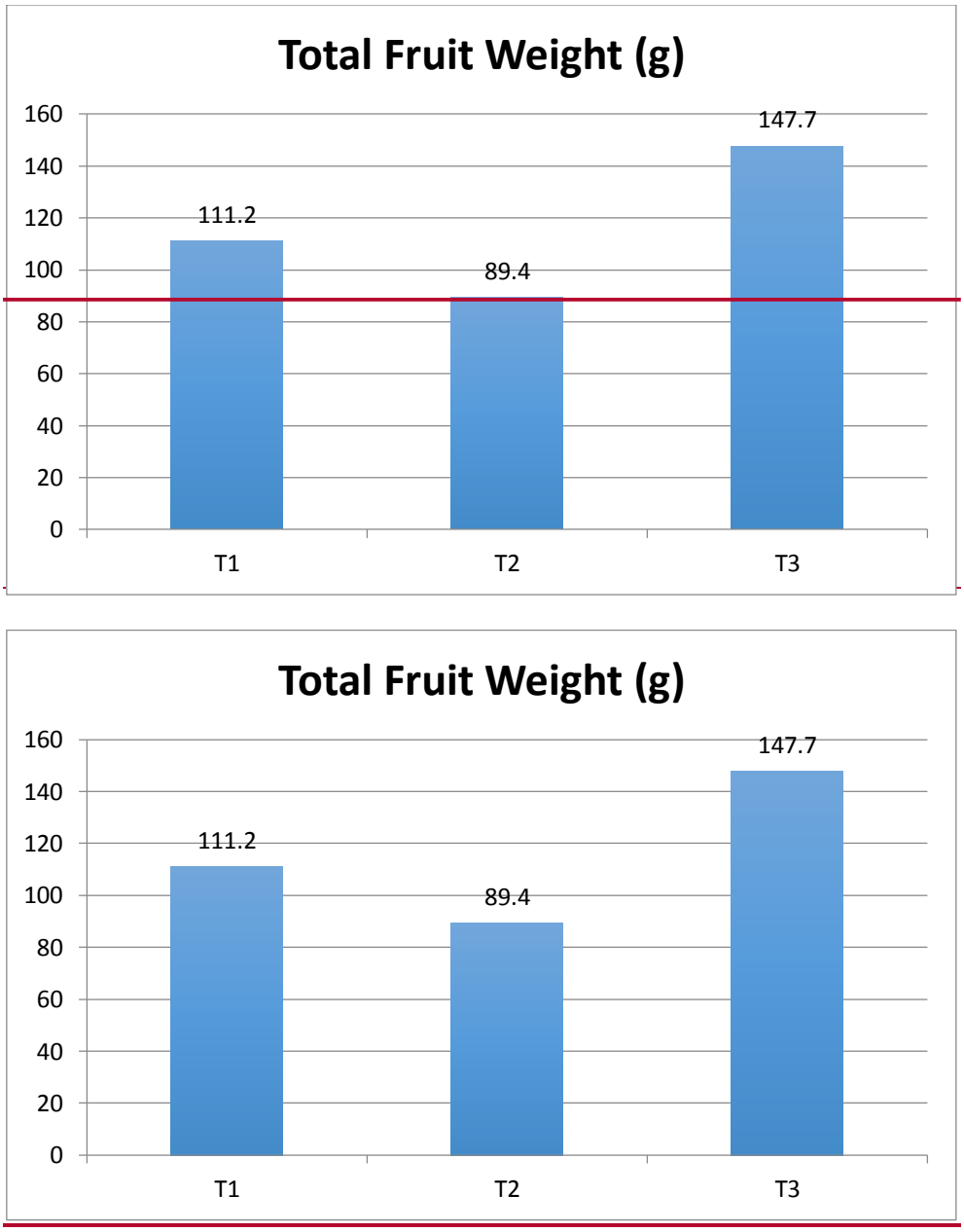

Chart 2. Total Fruit Weight Averages for all groups.

Staining:

When viewed under the dissecting scope, there were no visual signs of mycorrhizal colonization as no present fungal structures had been stained blue (Figure 5). Compound microscope analysis of one T1 species showed slight signs of fungal structures, but was

ultimately determined as insufficient signs for colonization (Figure 6).

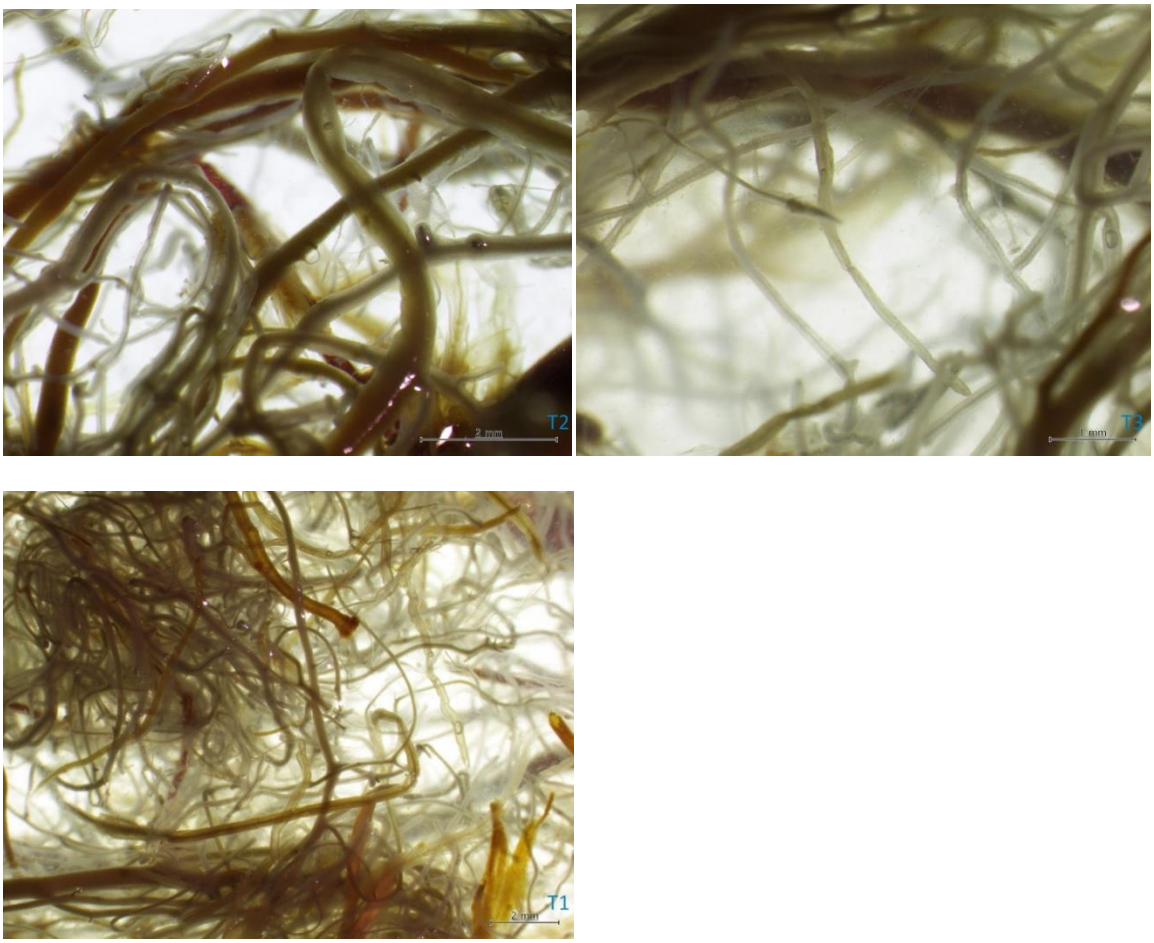

Figure 5. Subjects from all groups exhibited no evident signs of colonization when viewed under a stereo microscope.

Figure 6. T1 showing slight signs of fungal structures when viewed under a compound microscope.

Discussion

Our results suggest that both the single and mixed species commercial mycorrhizal products were unable to colonize the host strawberry plants. Neither of the shoot, root or fruit weight data sets indicated any statistical significance regarding enhanced health as a result of mycorrhizal symbiosis. Fungal staining and the subsequent microscopic analysis further confirmed the absence of colonization and could be the result of many factors.

The concept of fungal species being host specific may explain why neither treatment groups exhibited colonization, and that the genus *Glomus* (the fungus applied in both commercial products inoculum) is not compatible with *Fragaria*. Substituting either a different host or fungal specie/s into the same substrate and nutrient concentrations would confirm this theory. However, mutualism requires certain biological and physiological principles for initiation and could provide further complications beyond host-specificity.

Substrate characteristics such as the fertility level are known to effect colonization as the phosphorous (P) concentrations in particular could have exceeded the parameters of acceptable P. In a study which observed the efficacy of mycorrhizal applications in various growth medium supplied with 2 levels of soluble P, (Bierrman and Linderman, 1983) found that subjects inoculated in mineral soils with low P levels provided extensive colonization, enhanced growth and greater P concentrations in plant tissue (suggesting greater uptake by the host), but no observable growth response when high levels of soluble P were applied. Conversely, the soilless peat substrates provided minimal colonization and no enhanced growth at both low and high p

levels and supports the reasoning that the fertilizers of this experiment were too high in P and potentially inhibited colonization.

Sterilized, soilless media was used in this experiment to mimic common greenhouse production practices, as the industry often relies on these substrates for being disease and weed free as well as their consistency for repeatable results with high fidelity. The resulting obliteration of microbes could be a factor for our failed colonization, as research indicates that a community of mixed microbial species play a large role in the biological principles which work to dictate symbiosis (Labbe *et al*, 2014). Certain rhizospheric bacteria strains, known as mycorrhizal helper bacteria (MHB), have been determined to facilitate a higher probability for colonization; MHB work to increase spore germination and stimulate fungal growth as well as lateral root formation (Dames and Risdale, 2012) (Frey-Klett *et al*, 2011).

To determine if our colonization was confounded by these parameters, future experiments should substitute the media with an unsterilized mineral soil-based substrate. A more extensive experiment should incorporate additional inoculates which contain varying MHB species. Alternatively, to determine if high P levels inhibited symbiosis, substrate nutrient concentrations should be measured in addition to measurements taken after every fertilizer application.

Throughout the duration of the present experiment, certain greenhouse characteristics were discovered to be potential limitations by providing non-uniform environmental conditions like temperature, air flow and relative humidity. The degree of these factors varied between plants largely due to their spatial location and regardless of treatment type. The plants exhibited differential responses to environmental conditions depending on their proximity to the greenhouse control systems, which were intended to maintain these conditions evenly. While no

quantitative data was measured to confirm this, the most apparent sign of this was observed when plants closest to the cooling system yielded denser fruit with healthier color and seed distribution, while those on the opposing end yielded no fruit. This could be indicative of the greenhouse environmental conditions causing certain plants to undergo more stress over others and distort the data interpretation if colonization had occurred as a result.

Conclusion:

The results of this experiment suggest the commercial products MycoUp and MycoApply were ineffective in establishing mycorrhizal colonization between *Glomus* fungi and *Fragaria* x *annassasa* 'Albion'. The limiting factors believed to inhibit successful symbiosis were the source of the growth media, the presence of microbial diversity, substrate fertility levels, the environmental conditions of the greenhouse and which fungi or host species those factors will limit. The inclusion of mycorrhizae into the greenhouse industry should be based on the ability to manipulate these limiting factors, so that the advantages gained by the crop are repeatable and with high fidelity. While the mechanisms for mutualism are understood in theory, the practicality of large scale mycorrhizal applications can't be proven as viable until predictable success can be established.

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