EFFECTS OF PLANT GROWTH-PROMOTING RHIZOBACTERIA AND FUNGI ON STRAWBERRY PLANT HEALTH, FRUIT YIELD, AND DISEASE SUSCEPTIBILITY

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ABSTRACT

Effects of Plant Growth-Promoting Rhizobacteria and Fungi on Strawberry Plant Health, Fruit Yield, and Disease Susceptibility

Keen Maher

Studies on plant growth-promoting rhizobacteria (PGPR) and fungi (PGPF) as biostimulants have shown significant positive effects on plant health, fruit yield, or pest management. However, very few published studies to date have been specific regarding their effects on strawberries (*Fragaria* × *ananassa*), particularly on soilborne disease prevalence in organically grown strawberries. Empirical data on the results of using these products in commercial growing applications under various conditions would be highly valuable, especially for organic growers who have limited synthetic chemical pesticides, herbicides and fertilizers registered for use. The objective of this study is to evaluate the efficacy of biostimulant supplementation on strawberries for improving fruit yield, fruit quality, and plant health in both high-tunnel, open-sided 'hoophouse' and field conditions.

This study consisted of two research projects. The first project investigated the effects of commercially available PGPR-based biostimulant products on strawberry plant health. The three products contained differing proprietary combinations of PGPR, primarily from the *Bacillus* and *Lactobacillus* genera. Plants were grown in two different soil types: sandy and clay, in order to investigate the effects of biostimulant supplementation in different soil conditions. In fall of 2018, 160 'Monterey' strawberry plants were grown in an outdoor hoophouse in 3-gallon pots. Plants were either treated monthly with a single bacterial biostimulant product (EM-1, Accomplish LM, or Armory), or left untreated as a control. Plants were grouped into 20 blocks, each block comprised of 8 plants (each of the four treatments replicated in both soil types). Fruit yield (g), fruit sugar content (Brix), and leaf SPAD absorbance levels were measured weekly from January 27 to June 26, 2019. The treatments tested had no significant effects on fruit yield, leaf SPAD absorbance or Brix; soil type, however, did significantly impact fruit yield, with higher yields in sandy soil.

The second project was a field trial beginning in spring of 2020, in collaboration with Rutiz Farms in Arroyo Grande, CA, involving a total of 480 'Chandler' strawberry plants. The farm is organically managed and has a history of soilborne diseases, including *Verticillium dahliae*. These plants were either treated monthly with one of three microbial biostimulant products: a product containing a proprietary strain of *Trichoderma harzianum* biocontrol fungus (TrichoSym), and two of the same PGPR-based products used the previous year (Accomplish LM and Armory); or left untreated as a control. The experiment was laid out in a randomized complete block design with four blocks, with each block consisting of 4 plots for each of the 4 treatments; each plot contained 30

plants. Fruit yield (g) per plot was measured weekly throughout the 2020 growing season and phenotypic disease incidence was measured biweekly. Soil samples were taken at three different points throughout the season, cultured on selective media, and analyzed to obtain estimates of *V. dahliae* colony-forming units (CFU) per gram soil. The treatments tested had no significant effect on fruit yield, phenotypic disease incidence, or *V. dahliae* CFU/g soil. The results are inconclusive as to whether this lack of effect is due to viability of the products themselves, ineffective application techniques resulting in lack of rhizosphere colonization, or some combination of these. Further research is needed to determine whether or not supplementation with microbial biostimulants can produce reliable, beneficial results in strawberries.

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CHAPTER 1

Introduction

Many microbial species live in the rhizosphere of plants and establish symbiotic relationships with these plants, helping to increase nutrient availability, fixing nitrogen, increasing root surface area, and providing protection from disease (Vessey, 2003). Many different microbial strains occur naturally in compost and soil (Chandna et al., 2013). Beneficial soil microbes can be further subdivided into plant growth promoting rhizobacteria (PGPR), or plant growth-promoting fungi (PGPF). Products containing blends of these beneficial microbes are often commercially referred to as biostimulants. Microbial biostimulant products may be an individual strain of PGPR or PGPF, a blend of several strains from either of these categories, or a mixture of both PGPR and PGPF. Before growers invest money and time into the purchase and application of these products, it is important to determine how microbial biostimulants affect agricultural crop health, vigor, and disease susceptibility.

Microbial biostimulants represent one potential alternative to toxic, ozone-depleting fumigant chemicals, such as methyl bromide, that have traditionally been used to manage a wide variety of soil pathogens. They also may have the potential to reduce dependence on synthetic fertilizers. Groundwater contamination from synthetic nitrate fertilizers is an issue of increasing concern in modern agriculture, as groundwater nitrate levels steadily increase and other environmental toxins continue to accumulate (Spalding and Exner, 1993). Many PGPR strains have nitrogen-fixing abilities (Antoun et al., 1998; Anter et al., 2003), giving plants access to bioavailable nitrogen without any additional inputs. Other strains have been shown to increase plant uptake of phosphorus (Rodriguez

and Fraga, 1999) and iron (Vessey, 2003). Biostimulants made from these or similar PGPR strains could potentially be used to convert existing, recalcitrant soil nutrients into plant-available forms for uptake by plant roots. More research should be done on the potential of microbial biostimulants to improve plant health, as well as crop yield.

Additionally, there is a need to further investigate how these products can improve plants' resilience to a variety of stress conditions, including salt stress, drought stress, adverse pH levels, and soilborne diseases. Studies of this nature are typically only conducted on a single test crop, and as such, the results may not be applicable to other crops with different physiologies. The effects of microbial soil supplements may vary depending on the crop they are tested on, as different plants have unique morphology, environmental preferences and nutritional needs (Vessey, 2003). Very few studies to date have examined the effects of PGPR or PGPF supplementation on strawberry (*Fragaria* × *ananassa*) plants.

The objective of this research is to evaluate the effects of four different microbial biostimulant products, composed of various strains of plant growth-promoting rhizobacteria (PGPR) and plant growth-promoting fungi (PGPF), on the health, fruit yield, fruit quality, and soilborne disease status of strawberry plants grown in two different soil conditions.

CHAPTER 2

Literature Review

Research over the past half century has revealed a new class of compounds called biostimulants that can improve plant growth and plant physiology without requiring the addition of synthetic nutrients or pesticides. Biostimulants are derived from a variety of sources, and some of the most promising are microbial inoculants. Certain species of soil microbes, both bacterial and fungal, have been shown to decrease the incidence of fungal wilt diseases and beneficially alter crop vigor and yield. These microbes are generally either plant growth-promoting rhizobacteria (PGPR) or fungi (PGPF), both of which form obligatory relationships with the rhizosphere of plants. The objective of this literature review is to evaluate the current status of research on agricultural biostimulants, particularly beneficial microbial inoculants, and their potential for improving plant growth, yield, stress tolerance and disease resistance and management in strawberry cultivation.

2.1 Strawberry industry

2.1.1 Current issues in California

California is by far the nation's largest strawberry producer, growing a crop worth over $2.5 billion annually and accounting for over 90% of all US strawberry production; current California strawberry production acreage is approximately 36,480 acres, with 12.8% of this acreage (approximately 4,680 acres) managed using organic practices (California Strawberry Commission, 2020). In conventional strawberry production, growers have traditionally relied heavily upon the fumigant methyl bromide (also known as bromomethane) to control soilborne diseases, such as Verticillium wilt, Fusarium wilt,

and Macrophomina crown rot; weeds; and nematodes (Fennimore et al., 2008). The vast majority of California strawberry cultivars have been found to be highly susceptible to infection by *V. dahliae* (Shaw et al., 1996). *Verticillium* is a mitosporic, vascular-colonizing pathogenic fungus that can survive in soil for over 14 years, causing a variety of vascular wilt diseases in a number of crop plants (Carrero-Carrón, 2018).

Methyl bromide soil fumigation has proven to be an indispensable tool in California strawberry cultivation for controlling Verticillium wilt and other soilborne pathogens. An estimated 3.2 million pounds of methyl bromide were used to fumigate California strawberry fields in 2004 alone, and its application has been shown to increase strawberry yields by up to 94% annually; however, due to its widely known detrimental effects on the stratospheric ozone layer, methyl bromide is now completely banned in US agriculture (Fennimore et al., 2008).

Growers have begun to seek out alternatives to fumigants. A growing body of research on improved cultural management practices and more genetically resistant strawberry cultivars exists (Holmes et al., 2020). Certain strawberry cultivars, such as Camarosa, are fairly resistant to Verticillium wilt, and research has shown that strawberry plants exhibit polygenic resistance to the disease, rather than single-gene resistance; for breeders, this means resistance to *V. dahliae* is harder to achieve but more durable than in other crops with single-gene resistance (Gordon et al., 2006). Many strawberry cultivars are bred for pathogen resistance; however, one issue is that there are several distinct soilborne pathogens that infect strawberries, and each cultivar exhibits a differential resistance to these pathogens, and so a cultivar may be resistant to one pathogen but highly susceptible to another (Fang et al., 2012). Another promising alternative is the

usage of metam sodium, both alone and in combination with soil solarization (Hartz et al., 1993).

Some research has shown that certain microbial inoculants can significantly reduce the incidence of fungal wilt diseases (Gul et al., 2013; Senthilraja et al., 2012). Research done on olive (*Olea europaea*) trees, which are susceptible to vascular wilt caused by *V. dahliae,* found that application of *T. harzianum* GFP22 significantly reduced the extent of *V. dahliae* growth and the percentage of *V. dahliae* colonies recovered from vascular tissue of olive plants (Carrero-Carrón et al., 2018). However, very little research currently exists on the efficacy of beneficial microbials in reducing fungal wilt diseases such as Verticillium wilt in strawberries.

2.2 Biostimulants

2.2.1 Formal definitions and categories of biostimulants

Biostimulants are defined as certain biologically active compounds, separate from fertilizers, that are applied to plants in small doses in order to promote growth, enhance yields, improve stress tolerance, or provide defense against pests (Kunicki et al., 2010; Vessey, 2003). For decades, researchers have been identifying different microbial species that establish symbiotic and associative relationships with plant roots in the rhizosphere, and many companies are now marketing biostimulant compounds and their derivatives as biofertilizers or biocontrol agents for controlling pests and pathogens in agricultural crops (Vessey, 2003). The different categories of biostimulants include plant hormones, protein hydrolysates and amino acids, humic and fulvic acids, algal extracts, arbuscular mycorrhizal fungi, and plant growth-promoting rhizobacteria (Du Jardin, 2015).

2.2.2 Current research

Many biostimulant research studies have been conducted on a wide variety of agricultural crops; a large portion of them involved proteinaceous biostimulant products, often derived from fish. Protein hydrolysate-based biostimulant treatment has been linked to increased fruit yield in tomatoes (*Solanum lycopersicum*) and apricots (*Prunus armeniaca*) (Almaghrabi et al., 2012; Tarantino et al., 2018) and with increased dry matter yield in lettuce (*Lactuca sativa*) (Xu and Mou, 2017). Microbial biostimulants have been researched as well, though not as extensively. A rhizobia-based biostimulant treatment was correlated with increased auxin production, as well as increased dry matter yield in radishes (Antoun et al., 1998).

2.2.3 Microbial biostimulants

The category of microbial biostimulants can be further divided into PGPR and PGPF. Both have demonstrated the ability to increase nutrient uptake and nutrient use efficiency in a variety of different ways. PGPR-based biostimulants have also been linked to increased resistance to various phytopathogens. These PGPR increased resistance to *Fusarium* wilt in cucumbers (Gul et al., 2013), to cucumber mosaic cucumovirus in tomato (Zehnder et al., 2000), and also decreased nematode (*Meloidogyne* spp.) egg production in infected tomato plants (Almaghrabi et al, 2012). Plant growth-promoting fungi, notably strains of the soilborne and filamentous fungus *Trichoderma* spp., can develop a mutualistic association with plant roots leading to greater nutrient uptake, competition with pathogens, and improved plant defenses (Poveda et al., 2019). Both plant growth-promoting bacteria and fungi have been shown to confer a protective effect

under stress conditions by lessening the degree of damage caused by the buildup of reactive oxygen species (ROS) in plant cells (Mastouri et al., 2010).

2.3 Microbial biostimulants as biofertilizers

2.3.1 Biofertilizer effects of PGPR

Some plant growth-promoting rhizobacteria form symbiotic relationships with plant roots, living in the soil around roots or as endophytes inside root tissue (Glick, 2012). *Rhizobium* spp. form obligate relationships with legume roots, fixing atmospheric nitrogen into ammonia within the nodules on the plants' roots; legume seeds are commonly sold precoated with rhizobia to ensure thorough root inoculation for optimal plant health (Zahran, 1999). Plant growth-promoting rhizobacteria can directly promote plant growth by facilitating nutrient uptake (as in the rhizobia-legume symbiosis) or by altering phytohormone levels; or they can indirectly affect plant growth by working as biocontrol agents, diminishing the inhibitory effects of pathogens in the rhizosphere (Glick, 2012). Many other bacterial species can fix nitrogen, such as *Azospirillum.* Other species can solubilize mineral phosphate through the production of organic acids (Rodríguez and Fraga, 1999). Esitken et al. (2010) found that soil supplementation with a blend of *Bacillus* and *Pseudomonas* strains increased leaf phosphorus content in strawberry plants, and in turn increased fruit yield as well. Iron, another important plant nutrient that is a key component of chlorophyll, is abundant in most soils, but in soils is found predominantly in various mineral forms, most commonly as iron oxides and hydroxides, which have very low solubility and are therefore unusable by plants (Vansuyt et al., 2006). Some microbes secrete siderophores, which chelate mineral iron into soluble

compounds that can then be taken up by the cell. Beyond assisting with nutrient acquisition, many PGPR can also modulate certain plant hormone levels.

Some strains of PGPR have been found to produce plant growth hormones called gibberellins. For example, in one study on Chinese cabbage (*Brassica rapa*), crown daisies (*Glebionis coronaria*) and cucumbers (*Cucumis sativus*), application of a solution containing the PGPR *Acinetobacter calcoaceticus* significantly increased shoot length, plant height and plant dry weight in all three plants (Kang et al., 2008).

Another class of growth-promoting phytohormones synthesized by many PGPR strains are auxins, specifically indoleacetic acid (IAA), which influences cell division, seed germination, vegetative growth, root development, photosynthesis, stress response, and many other physiological processes (Tsavkelova et al., 2006). Research has shown that IAA produced by rhizobacteria can increase host plant root length and surface area; at the same time, IAA can increase plant root exudate production, which serves as a source of energy for these rhizosphere microbes (Glick, 2012). Other strains of PGPR which produce the enzyme 1-aminocyclopropane-1-carboxylate (ACC) deaminase can indirectly promote plant growth by decreasing endogenous levels of ethylene within plant tissues (Glick et al., 2007). Production of stress ethylene within plant tissues in response to pathogen infection may cause more damage to the plant than the pathogen itself; ethylene production negatively affects plant growth and yield in numerous ways, including premature fruit ripening and flower wilting, promotion of leaf abscission and senescence, and inhibition of root elongation and root nodule formation (Glick et al., 2007).

2.3.2 Biofertilizer effects of **Trichoderma** *fungus*

Much like the beneficial rhizobacteria, many PGPF species can improve crop plant health through a variety of mechanisms. It is thought that plant root colonization by the biocontrol fungus *Trichoderma* can augment nutrient uptake and improve nitrogen use efficiency, potentially leading to increased crop yield (Harman et al., 2004). Various *Trichoderma* spp. have been shown to solubilize plant nutrients in soil, increase photosynthetic rate and leaf CO_2 uptake, enhance Fe and P availability, and increase overall plant weight, leaf area, and shoot length (Altomare et al., 1999; Vargas et al., 2009; Yedidia et al., 2001). Two studies found that *Trichoderma* spp. can produce a variety of organic acids, including citric, fumaric, and gluconic acids, that may decrease soil pH and help to solubilize soil nutrients, including phosphates, magnesium, iron, and manganese, into forms more available for metabolic uptake by plants (Benítez et al., 2004; Harman et al., 2004). Another study by Porras et al. (2007) found that inoculating strawberry plant roots with a combination of two species of *Trichoderma* (*T. harzianum* and *T. viride*) significantly increased fruit yield, either alone or in combination with a soil solarization treatment.

Some secondary metabolites produced by *T. harzianum,* such as harzianolide and harzianopyridone, may promote plant growth via auxin-like activity at low doses, but confer an antimicrobial effect at higher concentrations (Hermosa et al., 2012). Different secondary metabolites with various antifungal properties are produced by different *Trichoderma* strains; these antifungal metabolites have been broken down into three main categories: volatile antibiotics, water-soluble compounds, and peptaibols (Ghisalberti and Sivasithamparam, 1991).

Nutrients are the primary benefit that plant hosts provide to *Trichoderma* in their root zones; mono-, di-, and polysaccharides excreted by roots provide nutrition to *Trichoderma,* which in turn may improve the plant's photosynthetic rate and various defense mechanisms (Hermosa et al., 2012).

2.4 Microbial biostimulants as anti-pathogenic agents

2.4.1 Anti-pathogenic effects of PGPR

Diseases caused by various phytopathogenic microorganisms are a major problem for the agricultural industry, especially the organic sector of the industry where the usage of common pesticides and fumigants is prohibited. Finding pathogen control methods that are approved for use in organic agriculture is an essential and immediate concern for growers. Plant growth-promoting rhizobacteria which produce ACC deaminase can reduce phytopathogen-inflicted damage by lowering plant stress response, namely ethylene production. This relationship allows plants to exist in the presence of pathogenic organisms without succumbing to ethylene-induced distress (Glick et al., 2007). Another way in which PGPR can control the growth phytopathogenic fungi is by competing with them for bioavailable iron. Bacterial siderophores have a higher affinity for iron than most fungal pathogens, and PGPR such as the *Pseudomonas* spp. have demonstrated an ability to control fungal pathogens including Phytophthora root rot and Fusarium wilt (O'Sullivan and O'Gara, 1992). Another study on peppers (*Capsicum anuuum*) found that phytophthora pepper blight, a disease caused by the oomycete *Phytophthora capsici* in combination with root rot fungi of the genera *Rhizoctonia* or *Fusarium,* was suppressed by soil inoculation with three PGPR strains that produced chitinolytic

enzymes, which break down chitin, an essential component of fungal cell walls (Kim et al., 2007).

Other strains of PGPR, including *Bacillus* and *Pseudomonas*, have been shown to trigger induced systemic resistance in some crops (Beneduzi et al., 2012). Some PGPR can also confer an antagonistic effect on soilborne fungal pathogens via the production of hydrolytic enzymes, such as chitinases, proteases, lipases, and glucanases, which have the ability to lyse fungal cells (Neeraja et al., 2010). Yet another mechanism of biocontrol exhibited by some beneficial PGPR strains is the production of antibiotic compounds which have the ability to control soilborne root diseases; these compounds include phenazines, cyclic lipopeptides, hydrogen cyanide, and phloroglucinols, among others (Haus and Défago, 2005).

One field trial in Greece found that 'Selva' strawberry plants (a cultivar known to be susceptible to Verticillium wilt) that were inoculated with both *V. dahliae* and the PGPR *Bacillus subtilis* (under the brand name FZB2411-WG) at planting and subsequently amended with a *B. subtilis* suspension one week after planting performed just as well as non-inoculated plants in terms of plant weight and fruit yield. (Tahmatsidou, 2006). However, the results of that study should only be considered with caution because the soil used was fumigated with methyl bromide prior to the root dip with *V. dahliae* suspension.

2.4.2 Anti-pathogenic effects of **Trichoderma** *spp.*

Phytopathogenic microorganisms can be bacteria, viruses, nematodes or oomycetes; however, the most common group of plant pathogens by far are fungal parasites, with over 20,000 known species (Cooper, 2007). A few of the most common,

and most problematic, phytopathogenic fungal species include *V. dahliae, Fusarium oxysporum* f. sp. *fragariae* and *Macrophomina phaseolina.*

Other, beneficial fungi, notably *Trichoderma* strains, can promote plant growth indirectly by parasitizing pathogenic fungi. Research by Woo et al. (2006) found that *Trichoderma* spp. release cell wall-degrading enzymes that break down the plant-parasitic host fungus cell wall; this releases compounds from the lysed host fungus cells, which may in turn trigger a defense response from the host plant. *Trichoderma* species have been shown to produce a variety of cell wall-degrading enzymes, or CWDEs, that exert an antifungal effect on a variety of not only fungal plant pathogens, but also fungus-like oomycete plant pathogens (Vinale et al., 2008). Secondary metabolite production is another important mechanism by which *Trichoderma* spp. exert a biocontrol effect on other, plant-pathogenic fungal strains. These secondary metabolites may be volatile, low-molecular-weight, nonpolar compounds such as 6-pentyl-α-pyrone; or polar antibiotics (Vinale et al., 2008).

Trichoderma may also induce both systemic and local plant resistance to several plant pathogens by colonizing plant root cells and synthesizing proteins and other compounds that may induce plant defense responses (Harman et al., 2004). Like many strains of PGPR, *Trichoderma* has been shown to promote induced systemic resistance via ethylene- and jasmonic acid-dependent pathways (Hermosa et al., 2012). The mechanisms by which these plant defenses are triggered has been investigated by several researchers. Roots of cucumber seedlings inoculated with *T. harzianum* T-203 were found to have stronger cortical and epidermal cell walls, and biochemical analysis of root cells showed evidence of increased activity of chitinase and peroxidase, suggesting the

development of some amount of systemic resistance (Yedidia et al., 1999). Certain proteins synthesized by *Trichoderma* during the root colonization process have been shown to act as microbe-associated molecular patterns, eliciting rapid defense responses from plants (Brotman et al., 2008).

Other research studies have investigated the effects of transgenically engineering *Trichoderma* genes into crop plants, notably the endochitinase gene *chit42*, isolated from both *T. harzianum* and *T. virens.* This introduced gene has been shown to confer resistance to *Alternaria*, *Rhizoctonia* and *Botrytis* strains in tobacco and potato (Lorito et al., 1998); to *Venturia inaequalis* in apple (*Malus domestica*) (Bolar et al., 2000); to *Alternaria brassicicola* in broccoli (*Brassica oleracea* var. *italica*) (Mora and Earle, 2001); to *Phoma tracheiphila* and *B. cinerea* in lemon (*Citrus limon*) (Gentile et al., 2007); and to *R. solani* in rice (*Oryza sativa*) (Shah et al., 2009), without noticeably affecting plant development or growth in any of these plants except apple, where decreased plant growth was observed. A final mechanism by which *Trichoderma* may antagonize other, pathogenic soil fungi is by simple competition for nutrients and space. Benítez et al. (2004) found that *Trichoderma* took up nutrients more efficiently than many other soil microbes observed in the study.

One study evaluating the effects of four different preparations of *T. harzianum* on strawberry infection by two fruit rot fungal species, *B. cinerea* and *Mucor piriformis*, found no significant effects on disease prevalence. A *Trichoderma* conidial suspension was applied weekly as a foliar spray to strawberries grown in a temperature-controlled, 12°C greenhouse; the authors posited that the *Trichoderma* preparations' lack of pathogen control efficacy may have been due to poor germination caused by a number of

potential factors including the cool greenhouse temperature, low relative humidity, or a lack of external available nutrients on leaf surfaces (Tronsmo et al., 1999). Another study compared strawberry plants either amended or not amended with *T. hamatum* pre-transplant and planted into soils that were either fumigated, amended with compost, amended with compost plus *T. hamatum* T382, or untreated; the study found that the *T. hamatum* maintained healthy, stable populations when added to compost, but not when applied to roots directly pre-transplant (Leandro et al., 2007).

These findings suggest that, when biological control using anti-pathogenic microbials in cropping systems is found to be effective, strategic application of these products is essential to ensure proper germination and continued survival of the microbes. Promising results from controlled-environment research studies cannot guarantee equivalent results in field-based conditions, and it is imperative that biocontrol fungi properly colonize root structures in order to enact their defense mechanisms (Hermosa et al., 2012).

2.4.3 PGPR research on strawberries

There are only a small handful of studies on PGPR supplementation in strawberry cultivation. A Serbian study looked at the effects of two liquid microbial inoculants, which were comprised of *Klebsiella planticol* (PGPR1) and *Azotobacter* spp. *, Derxia* spp., and *Bacillus* spp. (PGPR2), respectively, on greenhouse-grown 'Senga Sengana' strawberry plants (Pesakovic et al., 2013). They found that plants from both microbial treatments had significantly higher yields than the control group. Total acids and total sugars in fruit were increased in the treatments as well, though the effect was not considered significant. Another study from Turkey analyzed how three individual PGPR

strains (one *Pseudomonas* and two *Bacillus* spp.) affected organically produced strawberries. They found a significant increase in number of fruit per plant, as well as significantly higher leaf P and Zn content, compared to the untreated control plants (Estiken et al., 2009). An Egyptian strawberry study investigated the effects of Halex-2, a PGPR-based biofertilizer composed of three nitrogen-fixing strains (*Azospirillum, Azotobacter* and *Klebsiella*) in five different N-P-K fertilizer conditions. They found that the PGPR inoculant significantly increased fruit yield and total fruit sugar content, and posited that these effects were due to production of IAA and cytokinins by the beneficial microbes, leading to increased root hair branching and root nutrient uptake (Anter et al., 2003).

2.5 Conclusion

Many problems plague the agricultural industry in California, including phytopathogens and environmental concerns regarding synthetic fertilizers. The organic sector faces even more limiting rules and restrictions; this can be especially challenging when growing perennial crops, where disease populations can accumulate in soil over time. Even though strawberries can be grown as perennials, producing fruit for 3 to 4 years, most California growers plant strawberries as annuals to avoid recurring, progressive pathogenic diseases, including Verticillium wilt. However, in the advent of the ban on the fumigant methyl bromide, even conventional strawberry growers will be seeking out more sustainable, less toxic methods of controlling pathogenic fungal populations.

Microbial biostimulants may offer some promising prospects for phytopathogenic disease control, and for improving acquisition and efficient usage of vital plant nutrients.

As mentioned before, several studies have already been done on the effects of PGPR soil inoculants in strawberry cultivation. The objective of this study is to determine whether or not supplementation with microbial biostimulants has a measurable effect on fruit yield, overall plant health and mortality, or disease incidence in organically grown strawberries. This study is unique in that three different, proprietary PGPR blends are tested, and the interaction effect of PGPR supplementation and soil type is investigated as well.

CHAPTER 3

Project 1: Effect of Three Microbial Soil Supplements on Strawberry Plant Health Under Semi-Controlled Environmental Conditions

3.1 Introduction

While a number of studies have been conducted evaluating the efficacy of soil supplementation with beneficial 'biostimulant' microbes, there is a huge amount of variety in terms of test crops used, method of microbial product application, frequency of application, and methods of analyzing results. A thorough review of the literature for this study also did not reveal any studies comparing biostimulant supplementation on plants grown in different soil textures. Only a small handful of biostimulant studies on strawberries exist; of those, field trials are the standard, as opposed to trials on potted plants.

This project was designed to observe and analyze the effects of three different microbial soil supplements on the health and fruit yield of strawberry plants grown in two distinct soil textures. Plants were grown in individual pots in a high-tunnel, open-sided hoophouse; this allowed for protection from elements such as harsh winds or rain, while still maintaining ambient air temperatures and adequate ventilation. The study ran for approximately ten months, from planting until the plants had largely stopped producing fruit. The variables assessed included fruit yield (g) per treatment group, leaf SPAD absorbance (an indicator of chlorophyll content), and fruit Brix (sugar content).

The objectives of this project were:

1) to determine the effects of biostimulant supplementation with three different products on fruit yield, leaf SPAD absorbance, and fruit Brix of potted strawberry plants,

2) to determine the effects of clay versus sandy soil textures on fruit yield, leaf SPAD absorbance, and fruit Brix of potted strawberry plants, and

3) to determine if any interaction effect exists between biostimulant supplementation and soil texture on fruit yield, leaf SPAD absorbance, or fruit Brix of potted strawberry plants.

3.2 Materials and Methods

3.2.1 Experimental design

This study took place during the 2018-2019 growing season from October through July, located in a covered hoophouse in the Crops Unit of California Polytechnic State University (35.302103 N, 120.669695 W). In early December 2018, 160 bare-root 'Monterey' strawberry crowns (a day-neutral cultivar; Lassen Canyon Nursery, Redding, CA) were planted in 3-gallon plastic pots (11" x 11" x 9.5"; Growers Solution, Cookeville, TN) in an outdoor hoophouse in the Crops Unit at California Polytechnic State University, San Luis Obispo. All of the bare-root crowns were stored in a refrigerator at 40°F and were planted within one week after being received from the nursery; the crowns that were selected for planting appeared healthy and were all of a similar size, age and density, and none had any visible symptoms of disease. Half of the plants were planted in a clay-loam soil from the Cal Poly organic farm (35.3043 N, 120.6730 W), and half were grown in sandy soil from an organic farm in Santa Maria, CA (34.9076 N, 120.4836 W). All clay soil samples were uniform in texture and composition; all sandy soils were uniform in texture and composition. It was expected that some soilborne pathogens, such as the fungal pathogens *Verticillium, Fusarium* and *Macrophomina* might be present in the soils used in this study, as they are in many

agricultural soils in the central coast region of California. The soil from the Cal Poly organic farm had previously been tested for *Verticillium* and was estimated to contain approximately 51.4 CFU/g soil (Tubeileh and Stephenson, 2020); the presence of soilborne pathogens had not been previously diagnosed in the field where the sandy soil was taken from.

The plants were arranged on raised benches in a randomized block design, with 20 blocks consisting of 8 plants each, one plant for each treatment (see Table 3-1). All pots were amended with compost from Cal Poly and porcine bloodmeal (15-0-1) for an organic source of nitrogen prior to planting, and following planting, twice-monthly applications of granular organic fertilizer (6-6-6) were made (Vermicrop Organics, Olivehurst, CA) at a total rate of 12 g raw fertilizer per plant. All plants received the same amount of water, compost, preplant bloodmeal fertilizer, granular fertilizer, and mulch. Any variations in light level, tree canopy shading, or wind exposure were equalized by the complete block randomization design of the study.

3.2.2 Sample identification

There were eight treatments in this study, comprised of the three PGPR biostimulant treatments and a nontreated control, grown in each soil type. The three biostimulant products were EM-1, Accomplish LM, and Armory; see Table 3-2. Biostimulant treatments received fertilizer, compost, and the respective biostimulant product while the control plants were amended with only fertilizer and a 7.5 cm top layer of compost, which was then mixed throughout. Each treatment was replicated three times.

Table 3-1: List of eight treatment combinations of PGPR treatment and soil type.

PGPR TREATMENT	SOIL TYPE
NONE (CONTROL)	Clay
NONE (CONTROL)	Sandy
EM-1	Clay
EM-1	Sandy
ACCOMPLISH	Clay
ACCOMPLISH	Sandy
ARMORY	Clay
ARMORY	Sandy

3.2.3 PGPR soil inoculation

Immediately after planting, the biostimulant-treated plants were amended with one biostimulant product via an overhead drench application of 118 mL per plant. The products used and their ingredients and application rates are detailed in Table 3-2.

Table 3-2: Product information for PGPR treatments used in hoophouse project.

Product Name & Manufacturer	Active Ingredients	Concentration	Application Rate
EM-1 Microbial Inoculant (TeraGanix)	*Lactobacillus plantarum, L. casei, L. fermentum, L. delbrueckii, Bacillus subtilis, Saccharomyces cerevisiae, Rhodopseudomonas palustris*	1 million CFU/mL	1 oz/gal water (7.49 g/L)
Accomplish LM (Loveland Products)	*B. licheniformis, B. megaterium, B. pumilus*	1000 CFU/mL	1 oz/gal water (7.49 g/L)
Armory (Blacksmith Biosciences)	*B. subtilis, B. amyloliquefaciens, B. licheniformis, B. thuringiensis, Streptomyces griseus*	40 billion CFU/g	One teaspoon/gal water (1.11 g/L)

The first product used was EM-1 Microbial Inoculant, a liquid formulation manufactured by TeraGanix (South Alto, TX). Per the manufacturer's directions, the product can be applied just once per growing season, or up to once per week. The next product, Accomplish LM, is a liquid formulation manufactured by Loveland Products (Loveland, CO). The product manufacturer suggests a one-time application at a rate of 1 oz/gal (7.49 g/L) water. The last product, Armory, is a powder formulation manufactured by Blacksmith Biosciences (Spring, TX). The product manufacturer suggests a one-time application at a rate of one teaspoon/gal (1.11 g/L) water. Only one product was applied to each plant, at monthly intervals throughout the growing season. All plants were watered every other day by a drip irrigation system.

3.2.4 Data collection

Several plant growth and health parameters were monitored throughout the course of the study, from March through July of 2019. First, the weekly weight of fruit harvested from each treatment group was measured in grams, and the weekly average fruit yield was calculated for each treatment.

Twice per month, a Soil Plant Analysis Development (SPAD) meter was used to measure leaf SPAD absorbance, an indicator of chlorophyll content, from a subset of plants in each treatment. The SPAD meter measures the absorbance of certain wavelengths of light in order to determine the approximate amount of chlorophyll present in plant tissues. The SPAD meter used in this study was a SPAD-502Plus (Konica Minolta, Tokyo, Japan).

Fruit sugar content, or Brix, was measured in fruit from each individual fruiting plant in each treatment twice per month using a Brix refractometer (Weber Scientific,

Hamilton, NJ). When multiple ripe fruits were present on one plant, Brix readings were taken from each fruit and averaged together for that plant. A small drop of juice was squeezed from the fruit onto the lens of the refractometer, the protective screen was closed to seal in the liquid, and the refractometer was held up to the light to measure the Brix reading.

3.2.5 Statistical analysis

Statistical analyses were carried out using JMP (SAS Institute, USA) for analyses of variance (ANOVA). One-way ANOVA models comparing the four PGPR treatments were carried out for each of the independent variables: fruit yield weight, leaf SPAD absorbance, and fruit Brix level. These one-way ANOVA tests were done separately for each of the two soil types. Two-way ANOVA models incorporating both PGPR treatment and soil type were also carried out for each of the same variables mentioned above.

3.3 Results

Of the two factors analyzed in this trial, microbial treatment and soil texture, only soil texture had a significant effect. The biostimulants did not present any significant effects on weekly fruit yield. However, for each PGPR treatment, as well as across all treatments, the plants in the sandy soil had significantly higher weekly yields than the plants in clay soil ($P = 0.0005$). Here, the weekly fruit yield for each of the three PGPR treatments (EM-1, Accomplish and Armory) were 391 g, 376 g, and 384 g, respectively. The weekly fruit yield across all clay-soil treatments (67 plants total) was 279 g, while the weekly fruit yield across all sandy-soil treatments (69 plants total) was 489 g. The seasonal monthly fruit yields (per treatment, n = 20) for the three PGPR treatments (EM-1, Accomplish and Armory) were 1402 g, 1406 g, and 1419 g, respectively, versus 1429

g in the controls (Table 3-3). The seasonal monthly fruit yield for all clay-soil treatments was 4053 g, versus 7258 g for all sandy-soil treatments. Averaging for each individual plant gives a seasonal total of 60.5 g per plant in the clay soil treatments, and 105.2 g per plant in the sandy soil treatments.

Table 3-3: Monthly total and seasonal average 2019 monthly strawberry yields by treatment (n = 20). Values represent averages of all plants within each treatment ± one standard deviation.

Treatment	April total yield (g)	May total yield (g)	June total yield (g)	July total yield (g)	Seasonal average monthly yield (g)	Per plant average monthly yield (g)
Control, clay	1554 ± 87	365 ± 37	552 ± 56	1614 ± 359	1021 ± 198	60
Control, sandy	1573 ± 92	1190 ± 138	1735 ± 356	2846 ± 123	1836 ± 319	108
EM-1, clay	1413 ± 81	349 ± 53	652 ± 239	1913 ± 261	1082 ± 225	64
EM-1, sandy	1974 ± 161	973 ± 118	1163 ± 410	2776 ± 196	1722 ± 319	108
Accomplish LM, clay	1906 ± 74	596 ± 64	588 ± 81	1209 ± 301	1075 ± 144	57
Accomplish LM, sandy	2011 ± 146	932 ± 46	778 ± 209	3228 ± 135	1737 ± 335	97
Armory, clay	1236 ± 80	259 ± 43	307 ± 30	1697 ± 410	875 ± 219	62
Armory, sandy	1816 ± 169	939 ± 163	1828 ± 325	3270 ± 32	1963 ± 374	109
Clay average	6109 ± 82	1569 ± 53	2099 ± 120	6433 ± 301	4053 ± 196	60
Sandy average	7374 ± 140	4034 ± 116	5504 ± 321	12120 ± 138	7258 ± 332	105
Control average	1564 ± 87	778 ± 129	1144 ± 314	2230 ± 329	1429 ± 274	42
EM-1 average	1694 ± 128	661 ± 108	908 ± 314	2345 ± 260	1402 ± 280	42
Accomplish LM average	1959 ± 112	764 ± 64	683 ± 146	2219 ± 423	1406 ± 262	38
Armory average	1526 ± 133	599 ± 133	1068 ± 346	2484 ± 388	1419 ± 320	44
PGPR average	1726 ± 124	675 ± 103	886 ± 271	2349 ± 344	1409 ± 286	42

As shown in Fig. 3-1, seasonal marketable fruit yield was not significantly different between the PGPR-treated versus untreated plants (388 g/week versus 385 g/week; $P = 0.9613$). However, soil type did have a significant effect on seasonal fruit yield , with significantly higher yield in the sandy soil ($P < 0.0001$).

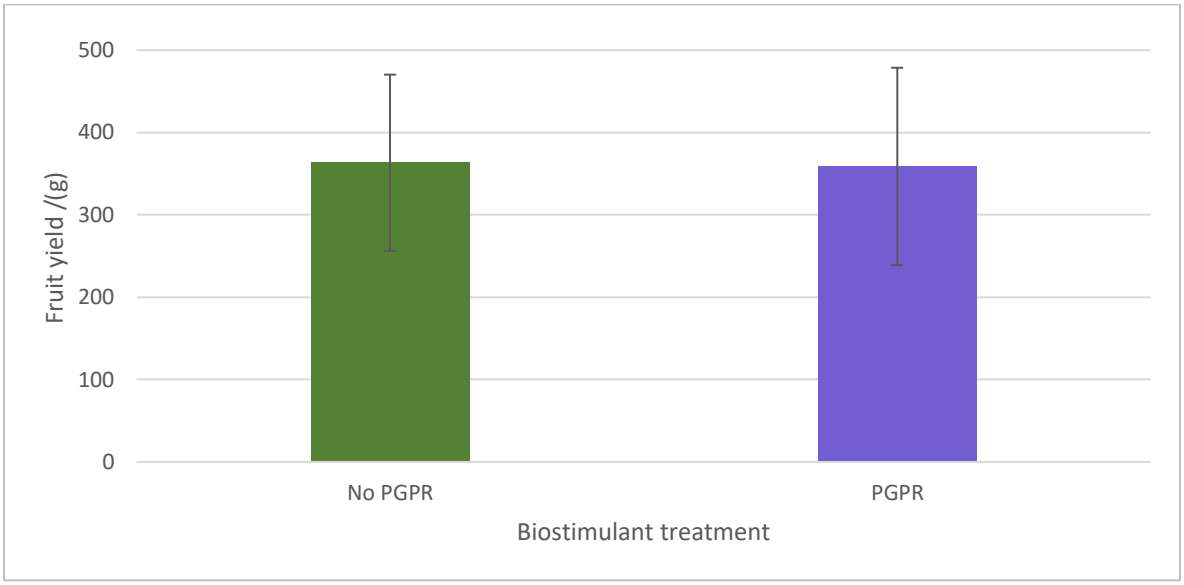


Figure 3-1: Seasonal average Monterey strawberry weekly fruit yield per plant (g), with or without addition of PGPR-based biostimulant product. Error bars represent one standard deviation of the mean.

The plants grown in sandy soil had significantly higher average fruit yields than those grown in clay soil (489 g/week versus 279 g/week), regardless of which PGPR treatment was applied (Figure 3-2; Figure 3-3).

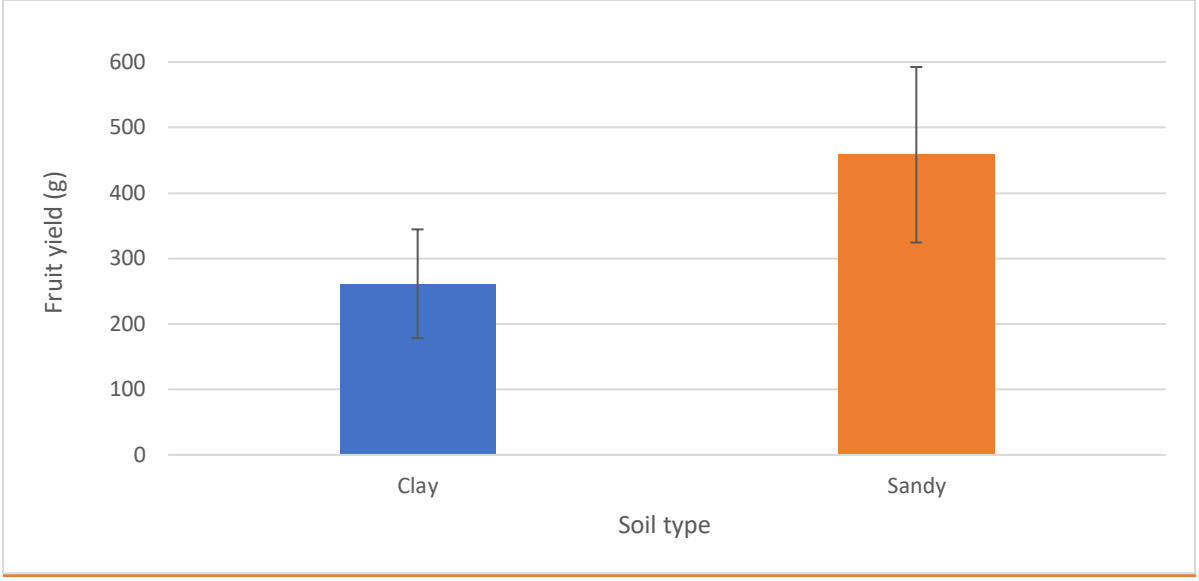


Figure 3-2: Seasonal average (n = 16) Monterey strawberry weekly fruit yield (g) by soil type. Error bars represent one standard deviation of the mean.

There was no significant difference in seasonal weekly fruit yield between any of the three biostimulant product treatments (EM-1, Accomplish, or Armory); or between any of the biostimulant treatments and the control.

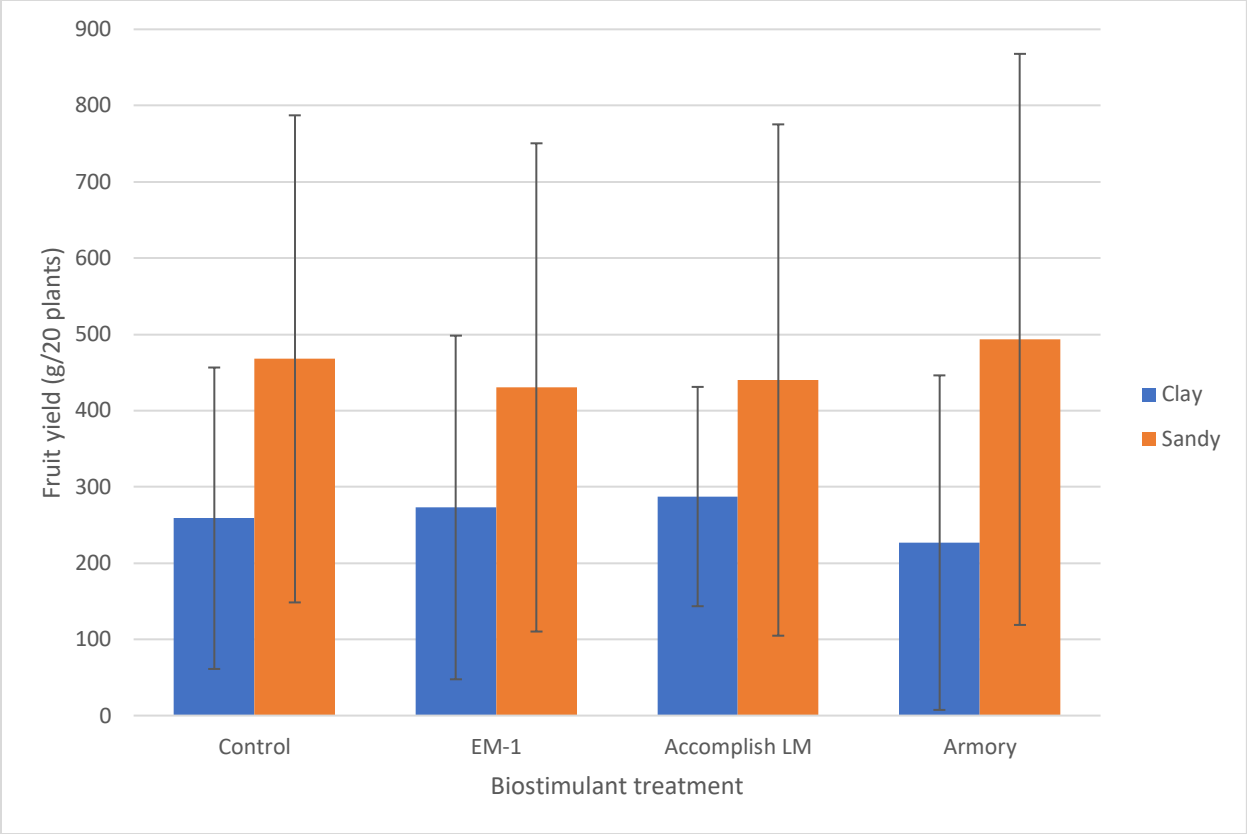


Figure 3-3: Seasonal average (n = 16) weekly fruit yield (g) by treatment. Error bars represent one standard deviation of the mean.

Cumulative seasonal fruit yield was able to reveal more trends in the data than the weekly fruit yield (Fig. 3-4). The standard error bars indicate that there were significant differences between the three biostimulant treatments groups (EM-1, Accomplish LM, and Armory); between the biostimulant groups and the controls; and between the clay and sandy soil types.

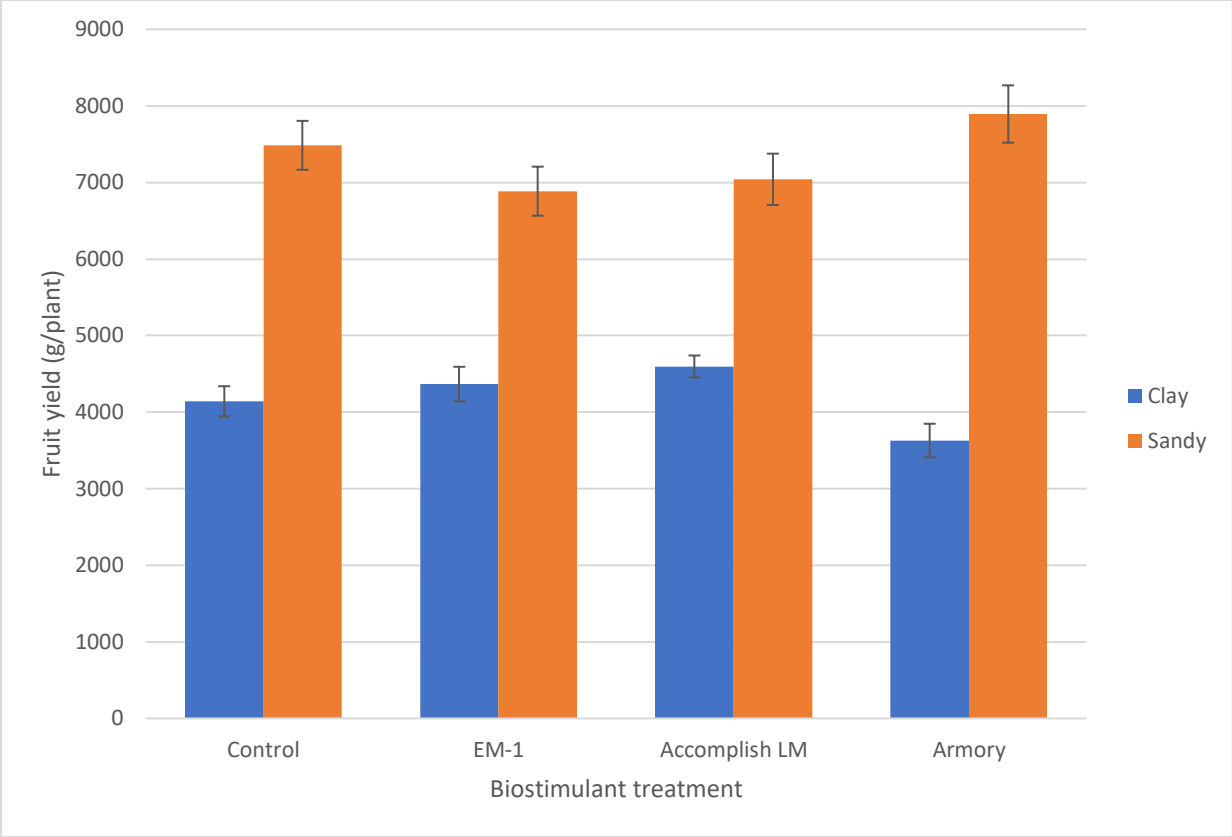


Figure 3-4: Seasonal cumulative fruit yield (g) by treatment. Error bars represent one standard deviation of the mean.

Some slight variations in Brix level were detected between treatments (Fig. 3-5), but these differences were not significant ($P = 0.314$). Soil type also did not significantly affect Brix levels ($P = 0.959$).

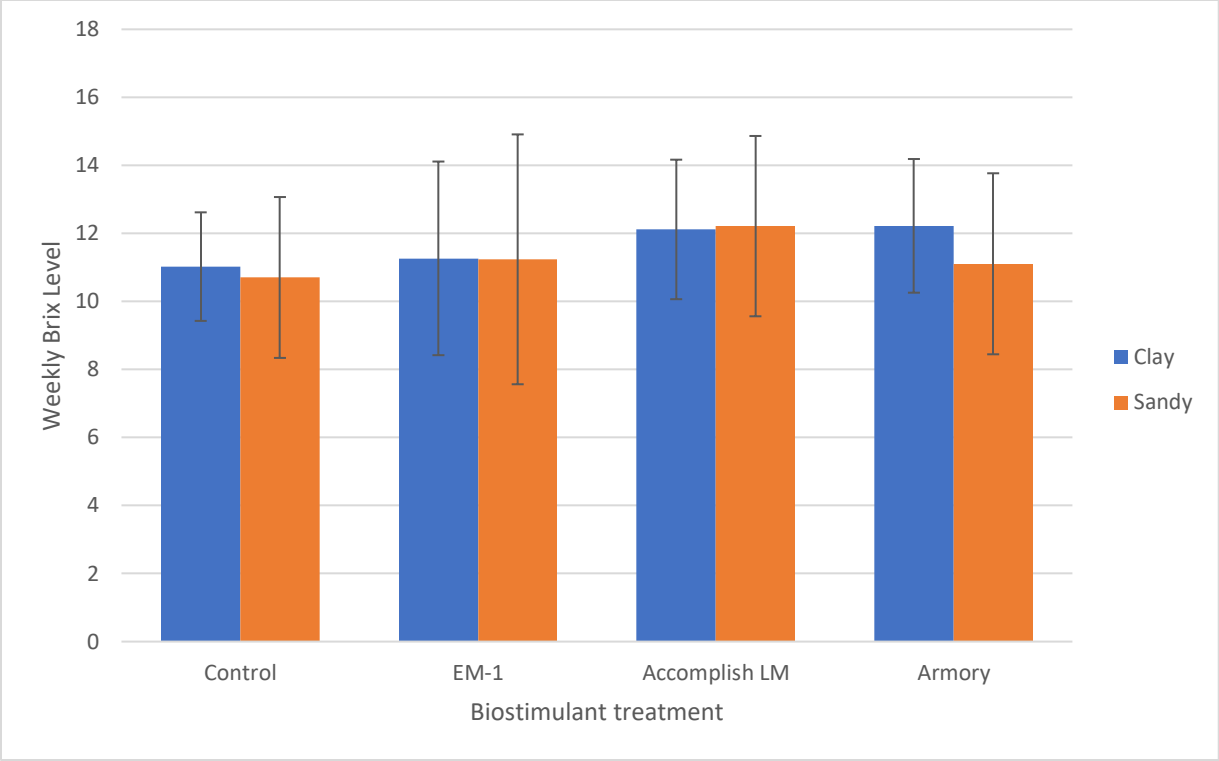


Figure 3-5: Seasonal average (n = 5) Brix levels in Monterey strawberry plants by treatment. Error bars represent one standard deviation of the mean.

Figure 3-6 shows the seasonal leaf SPAD absorbance levels by biostimulant treatment and soil type. There were no significant differences in seasonal average leaf SPAD absorbance between any of the biostimulant treatments, or between any treatment and the control ($P = 0.449$). There were also no significant leaf SPAD absorbance differences between treatments at any of the time points measured throughout the study. There was, however, a significant difference in leaf SPAD absorbance levels between the sandy soil and clay soil groups, with plants grown in clay soil having significantly higher leaf SPAD absorbance levels ($P = 0.035$).

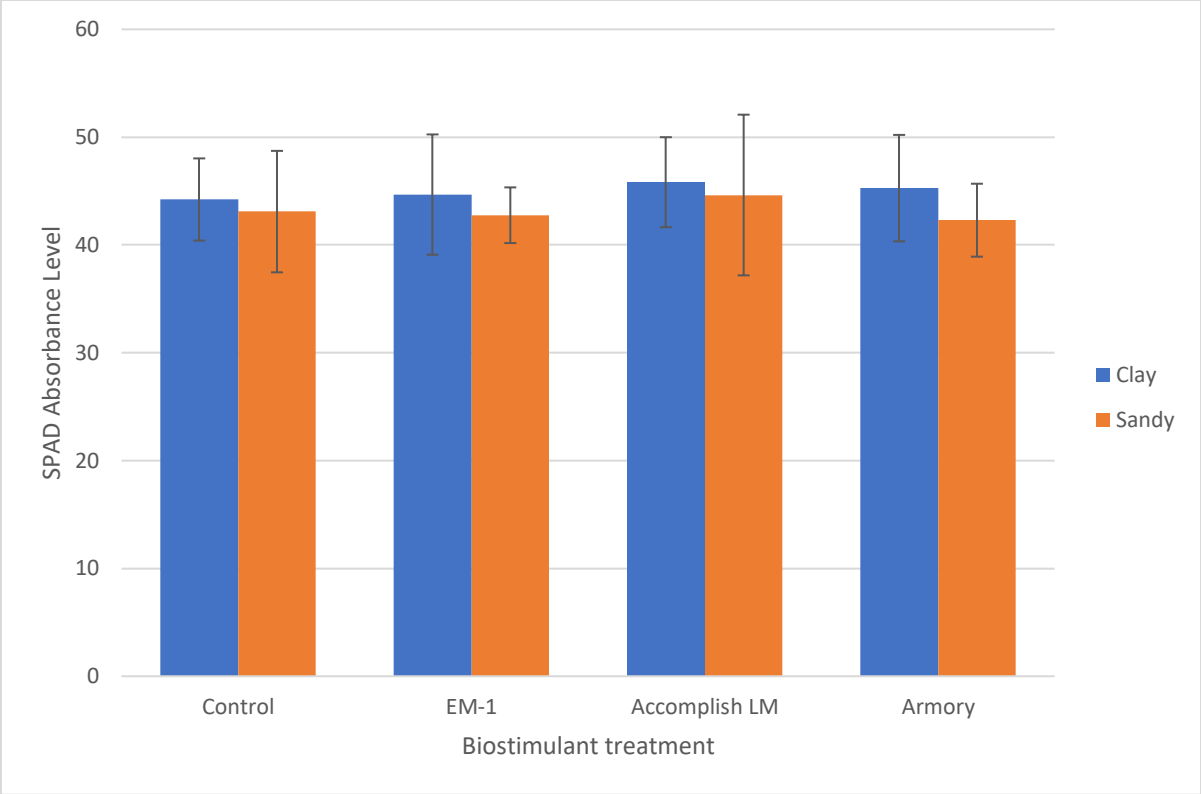


Figure 3-6: Seasonal average (n = 6) leaf SPAD absorbance levels in Monterey strawberry plants by treatment. Error bars represent one standard deviation of the mean.

No interaction effect between biostimulant treatment and soil texture was found to exist for fruit yield (P = 0.882), leaf SPAD absorbance (P = 0.858), or fruit Brix (P = 0.072).

3.4 Discussion

In this trial, the effects of two factors (PGPR biostimulant supplementation and soil type), as well as their interaction, on strawberry fruit yield, leaf SPAD absorbance (chlorophyll content), and fruit Brix (sugar content) were evaluated. In terms of soil type, the plants grown in sandy soil had significantly higher yields than those grown in clay soils. This finding agrees with previous knowledge that strawberries are known to prefer sandy soils as a growing medium; heavy clay soils are notorious for hampering growth,

encouraging soilborne disease, and impeding the proper drainage that strawberry plants need (Collier, 2012).

The results of this trial indicate that PGPR biostimulant soil supplementation does not significantly increase fruit yield in strawberries grown in pots in a hoophouse setting. The biostimulant products tested also did not significantly alter leaf SPAD readings or fruit Brix readings. It is possible that limited nutrient and/or substrate availability contributed to the inefficacy of these products in this trial.

One issue may have been that there was not sufficient organic substrate, such as compost, for the beneficial microbes to survive on, as was the case in the study by Leandro et al. (2007); ironically, that same study also found that *Trichoderma* biocontrol strains had a 60-fold greater survival rate in fumigated versus non-fumigated soil, presumably due to lack of competition from preexisting soil microbes. Further research should include measures to ensure that PGPR strains are still viable at multiple intervals after application, as well as additional pathology tests to determine the cause of death or stunting in diseased plants.

Another issue encountered during the course of this study is that application and storage instructions on some of the products tested were frustratingly vague. One product had no dilution information on the label and had to be manually calculated; another product had no information, on the label or online, on how to store the product, which required contacting a company representative to clarify. It is doubtful that the average strawberry grower would have the time or energy to bother with such details. This makes one wonder how much quality assurance testing has been done to guarantee that the microbial strains inside the container are in fact alive, viable and present in the listed

concentrations. Certain strains of PGPR or PGPF may well be highly beneficial to growing plants, but only if they are properly preserved and capable of surviving in both storage and in the plant's rhizosphere.

The results of this study are in agreement with some, but not all, previous studies of a similar nature. Blauer and Holmes (2019) tested a variety of microbial soil supplements and found that none of the *Bacillus*-based treatments resulted in significantly higher cumulative fruit yields. This corroborates the findings in this study, in which neither of the two *Bacillus*-based products tested produced any noticeable increases in fruit yield. Furthermore, researchers at both California Polytechnic State University and the University of Florida have tested dozens of different microbial biostimulant products over the course of a decade and have not found any that exerted significant beneficial effects on the crops tested (Holmes, 2020); they posit that lack of adequate rhizosphere colonization by the supplemental microbes may have been the fundamental problem. This parallels the conclusions mentioned by researchers who tested a foliar conidial suspension of *T. harzianum* on greenhouse-grown strawberries and found that the treatment had no effect on the prevalence of fungal fruit rot (Tronsmo et al., 2008). Although rhizosphere colonization is different from foliar colonization, poor germination of the biostimulant organisms is the same underlying problem posited by researchers. Further research is necessary to determine the most effective application techniques and timing for optimal PGPR/PGPF rhizosphere colonization and proliferation.

CHAPTER 4

Project 2: Effect of Three Microbial Soil Supplements on Strawberry Plants Under Field Conditions

4.1 Introduction

The majority of strawberries grown organically in the central coast region of California are field grown in soil that cannot be treated with fumigants for soilborne pathogen control. Field soil may contain a number of different microbial pathogens, which can wreak havoc on strawberry plants even in very low numbers (Tahmatsidou et al., 2006). Without many effective chemical control options, organic growers may depend on cultural or biological controls to reduce fruit losses due to pathogen infestation. Microbial supplementation with PGPR or PGPF presents one such biological control option for strawberry growers who are struggling with microbial pathogens in their soil.

This project aimed to observe and analyze the effects of three different microbial biostimulants, one PGPF-based and two PGPR-based, on the health and fruit yield of field-grown strawberry plants. The effect of supplementation with these microbial products on the *V. dahliae* pathogen load of the soil itself was also assessed at multiple time points throughout the course of the study. This study ran during the first half of 2020, from January through June, at which point the plants had largely stopped producing fruit. The variables tested in this study included fruit yield per treatment group, *V. dahliae* CFU/g soil, and individual plant disease status on a categorical scale from 0 to 4, which was then used to plot disease progress over time.

The objectives of this study were:

1) to determine the effects of biostimulant supplementation on fruit yield and plant disease progress over time in field-grown strawberry plants, and

2) to determine the effect of biostimulant supplementation on soil *V. dahliae* populations (CFU/g soil) in field-grown strawberry plants.

4.2 Materials and Methods

4.2.1 Site description

This study was conducted during the 2019-2020 growing season from November to July, located within a section of Rutiz Farms in Arroyo Grande, CA (35.105001 N, 120.598717 W). The soil is sandy loam and has a history of *Verticillium* infestation, especially towards the western end of the field, where this research study was located (see map in Appendix B). However, no soil tests to determine *Verticillium* concentration in the soil were conducted prior to the beginning of this trial. No strawberries had been grown on this particular section of the field for eight years prior; the previous year's crops on this field were oats and a variety of legumes. The soil was listed and shaped to create 48 cm wide raised beds (13-15 cm). The beds were covered with a black plastic tarp. A preplant chicken manure fertilizer (4-4-4; Perfect Blend Organics, USA) was mixed into the soil immediately before planting at a rate of 1,120 kg/ha. After planting, subsequent fertigation applications 14-0-0 liquid fertilizer (Westbridge Ag. Products, USA) were made at a rate of 11.2 kg/ha (10 lb/acre) every two weeks, for a total of 45.36 kg (100 lb) throughout the season, to supply a continuous source of nitrogen for growing plants.

Uniform, bare-root 'Chandler' strawberry crowns were transplanted in early November 2019 in double rows with plants spaced 12" apart. Plants were watered with

drip irrigation every five days, for four hours each time, resulting in a total rate of approximately 20,000 gallons of water per acre every five days. The ‘Chandler’ variety of strawberries is a University of California cultivar from 1983 that is June-bearing and known to be very susceptible to multiple soilborne pathogens, including *Verticillium* and *Macrophomina.*

4.2.2 Experimental design

Four adjacent rows of Chandler strawberry plants were further divided into four equally sized plots, for a total of 16 plots. It is assumed that any variations in light level, wind exposure, or ground sloping were equalized by the randomized complete block design and Latin square design of the study.

All soil samples were observed to be uniform in texture and composition. It was expected that some soilborne pathogens, such as the fungal pathogens *V. dahliae, F. oxysporum* and *M. phaseolina* would be present in the soil used in this study, as they are in many agricultural soils in central California; however, later pathology tests from the Cal Poly strawberry pathology lab on dead plants from this trial found no evidence of the presence of any of these pathogens, indicating that the plants had likely died from abiotic causes.

There were four treatments in this study, comprised of three microbial products and an untreated control. The PGPF product was TrichoSym, and the two PGPR products were Accomplish LM and Armory. Starting on January 27, 2020, the microbial inoculants were applied by hand to individual plants via a drench application of 118 mL (four ounces) per plant. Control plants were given the same volume of water without any treatment. Treatments were applied once every two weeks for the first two months, and

once per month thereafter. All three of the PGPR biostimulant products were applied according to package directions, and all were applied with equal frequency.

The study was carried out on a total of 480 plants, organized into four distinct blocks of 120 plants each. Each block was made up of four randomized plots, with one plot of each treatment per block (Appendix A). Each of these individual plots contained 30 plants and was approximately 6.1 meters long and 48 cm wide and raised 13-15 cm from ground level in the center.

4.2.3 Disease identification

All plants in each treatment were examined weekly for pathogen-related disease symptoms using the following rating scale: 0, no symptoms; 1, leaf puckering or curling just beginning; 2, 50% of leaves on plant appear puckered or curled; wilt symptoms just beginning; 3, 50% of leaves showing wilt symptoms; 4, a dead or severely stunted plant with almost 100% of leaves showing wilt symptoms (Fig. 4-1).

Figure 4-1: Examples of strawberry plants ranked on a disease severity scale from 0 to 4.

Disease severity values were calculated using Equation 1 (Yang et al., 1996):

$$\text{Disease severity (Y)} = \frac{\sum (\text{disease grade} \times \text{number of plants in each grade}) \times 100}{(\text{total number of plants})\ (\text{highest disease grade})}$$

For one block, or one treatment, the total number of plants = 120 and the highest disease grade = 4. Simplified equation:

$$\frac{\sum (\text{disease grade} * \text{number of plants in each grade}) * 100}{480}$$

Disease progress over time was measured for each plot using Equation 1 at each of six different time points throughout the study.

Composite soil samples taken in late January, prior to application of any biostimulant products, were chemically analyzed by A&L Western Agricultural Laboratories (Modesto, CA). The results of the chemical analysis are shown in Table 4-1.

Table 4-1: Soil chemical properties from samples taken January 27, 2020 from Rutiz Farms in Arroyo Grande, CA.

Block	OM % Rating	% K Saturation	% Mg Sat.	% Ca Sat.	% Na Sat.	pH	Cation Exchange Capacity (CEC)
1	1.1	2.4	23.6	70.9	3.2	7.4	5.1
2	1.2	2.8	23.2	80.0	3.0	7.5	5.2
3	1.0	2.6	23.7	70.5	3.3	7.5	5.1
4	1.0	2.7	23.3	70.9	3.1	7.5	5.1

On January 30, preliminary soil samples were taken and soil *V. dahliae* populations were assessed prior to the initial application of microbial products, and subsequent samples were taken on April 10 and June 26 in order to monitor soil *V. dahliae* populations. Soil samples from the area immediately surrounding plant roots were consistently taken from four selected plants within each plot; the location of the four plants was the same across every plot. After air drying in the laboratory for two weeks, soil samples (0.10 g each) were diluted 10X in deionized water, and 1 mL samples of this suspension were plated in triplicate on NP-10 selective media (Kabir et al., 2004; see Appendix C) and incubated in the dark for 15 days. After incubation, *Verticillium*

microsclerotia numbers were counted using a dissecting microscope at 10X magnification (Olympus Inc.), and the CFU per gram of soil were recorded.

4.2.4 Data collection

Marketable fruit yield harvested from each plot was measured weekly, and the average fruit yield was calculated for each treatment. Once per week, each individual strawberry plant was photographed and rated on a disease-severity scale of 0-4. The categories in this scale are the same as those described in section 4.1.2 (p. 29) and illustrated in Figure 4-1.

4.2.5 PGPR and PGPF soil inoculation

On February 1, 2020 (approximately 3 months after the strawberries were planted), the biostimulant-treated plants were amended with one biostimulant product via an overhead drench application of 4 ounces per plant. Product A was TrichoSym Bio, a liquid formulation manufactured by Symborg (Ventura, CA). The company guarantees 5×10^{11} CFU/L, and its active ingredient is the proprietary *T. harzianum* strain T78. The product is applied at a rate of 1 oz/gal (7.49 g/L) of water. Product B and Product C were Accomplish LM and Armory, respectively; the details of these formulations are listed in Table 3-2. Only one product was applied to each plant, at two-week intervals for the first two months, and then subsequently at monthly intervals throughout the growing season.

4.2.6 Statistical analysis

All statistical analyses were carried out using JMP 14 (SAS Institute, USA). The significance level (p-value) used for all data was 0.05. One-way ANOVA models comparing the four treatments were carried out for each of the independent variables: fruit yield weight, categorical disease rating, and *Verticillium* CFU/g soil. One-way

ANOVA models comparing the four blocks (rows) were also carried out for each of these same independent variables to look for differences between blocks. Two-way ANOVA models incorporating both biostimulant treatment and block were also carried out for each of the same variables mentioned above.

4.3 Results

None of the three biostimulant treatments resulted in significantly higher seasonal average strawberry yields than the control ($P = 0.9119$). Here, the seasonal average monthly fruit yield for the three treatments (TrichoSym, Accomplish LM, and Armory) were 378.4, 383.4, and 358.9 g plant^{-1}, respectively, versus 366.2 g plant^{-1} in the controls (Table 4-2).

Table 4-2: Monthly total and seasonal average 2020 strawberry fruit yield by treatment (n = 4) and by block (n = 120). Values represent averages ± one standard deviation.

Treatment/ Block	March total yield (g)	April total yield (g)	May total yield (g)	June total yield (g)	Seasonal average monthly yield (g)
TrichoSym	3020.40 ± 99.52	8384.00 ± 271.55	9560.60 ± 374.28	4373.80 ± 128.64	6334.70 ± 294.22
Accomplish LM	2886.50 ± 85.72	8446.90 ± 275.21	9481.30 ± 353.71	4415.80 ± 163.95	6307.63 ± 292.06
Armory	2815.60 ± 122.40	6277.60 ± 146.96	10199.50 ± 335.52	4832.70 ± 190.76	6031.35 ± 271.33
Control	2890.70 ± 78.92	7938.90 ± 162.96	8746.30 ± 275.05	4931.20 ± 221.80	6126.78 ± 240.99
Block 1	2682.00 ± 92.81	7853.30 ± 182.04	6610.30 ± 202.27	3141.40 ± 110.22	5071.75 ± 200.50
Block 2	3793.10 ± 112.83	9005.60 ± 232.15	14746.90 ± 299.60	5976.40 ± 159.11	8380.50 ± 330.17
Block 3	3191.50 ± 63.54	9156.20 ± 226.27	11022.90 ± 285.80	6038.40 ± 145.98	7352.25 ± 272.53
Block 4	1946.60 ± 74.23	5032.30 ± 163.26	5607.60 ± 152.29	3397.30 ± 122.58	3995.95 ± 158.61

Figures 4-2 through 4-5 display the trends in cumulative strawberry fruit yield, in weekly intervals, over the course of the growing season in blocks 1 through 4,

respectively. None of the biostimulant-treated groups had significantly higher fruit yields than the untreated control, based on cumulative yields ($P = 0.9119$). However, block location did appear to have a significant effect on cumulative seasonal fruit yield (Fig. 4-4; $P < 0.0001$), as well as on weekly average fruit yield (Fig. 4-5; $P < 0.0001$).

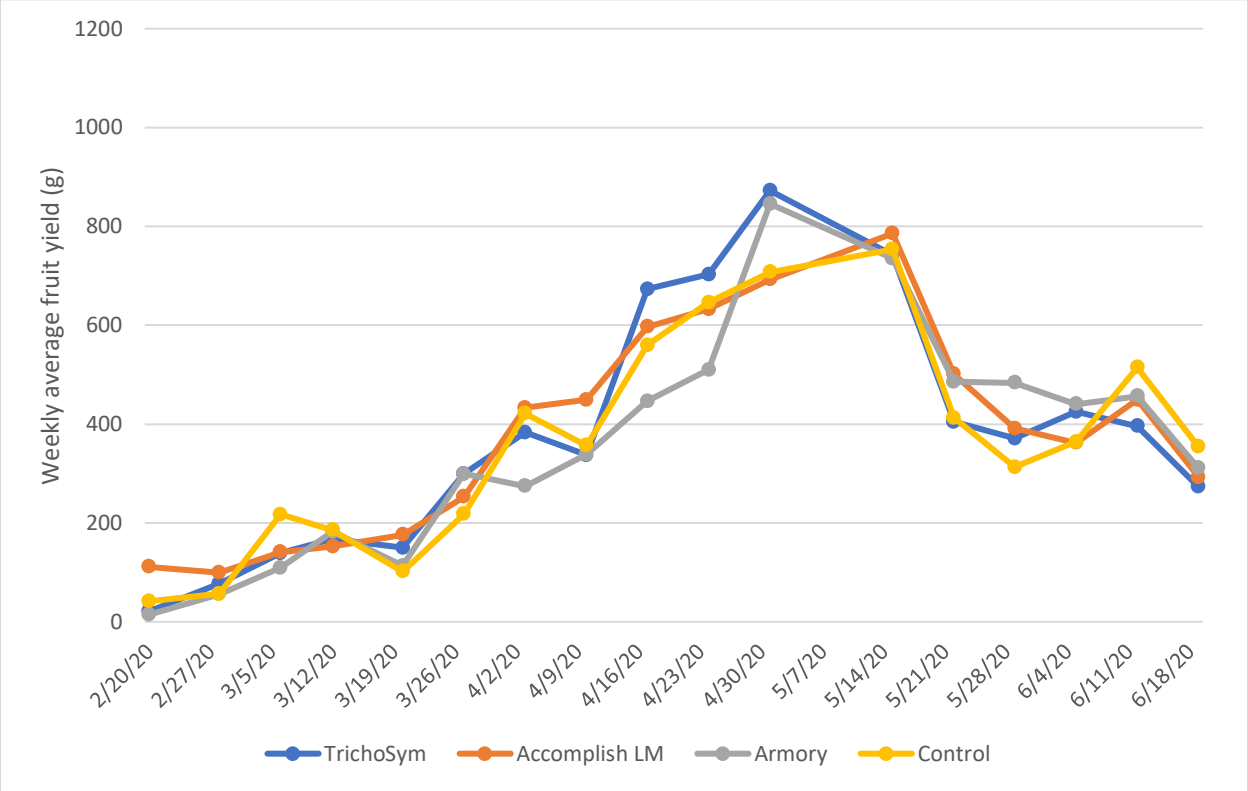


Figure 4-2: Chandler strawberry fruit yield by treatment over time during the 2020 growing season; different lines represent different treatments.

Cumulative seasonal fruit yield was significantly different ($P < 0.0001$) across each of the four blocks. Block 2 consistently showed the highest weekly average yields (n = 4, 439.7 - 544.3 g) with Armory-treated plot 2C reaching both the highest weekly average fruit yield (544.29 ± 404.5 g) and the highest cumulative seasonal yield at 9,253.0 grams. Block 3 had the second-highest weekly average fruit yields (n = 4; 375.1 – 498.5 g) and seasonal cumulative fruit yields (6,377.2 – 8,474.7 g) out of the four. The lowest weekly average yields were seen in block 4 (n = 4; 161.5 – 302.3 g), with

Accomplish LM-treated plot 4B yielding the lowest cumulative seasonal fruit weight of only 2,744.9 ± 115.1 g. These results suggest that there may have been preexisting, underlying differences in the microbial abundance and/or composition of the soil in these 4 different blocks. All blocks showed consistently low *Verticillium* CFU values prior to any product application, so another soilborne microbe, or possibly invertebrate activity, may explain these observed yield differences. All plants were watered, fertigated, and pruned of runners in the same manner, and all received roughly the same amount of sunlight each day, so none of those factors should have contributed to yield discrepancies between blocks.

Block location also caused significant ($P < 0.0001$) differences in weekly average strawberry fruit yield, although standard errors were quite large. Column location (vertical location within the Latin square layout) was also taken into account but was not found to have a significant impact on the weekly average fruit yield ($P = 0.2498$).

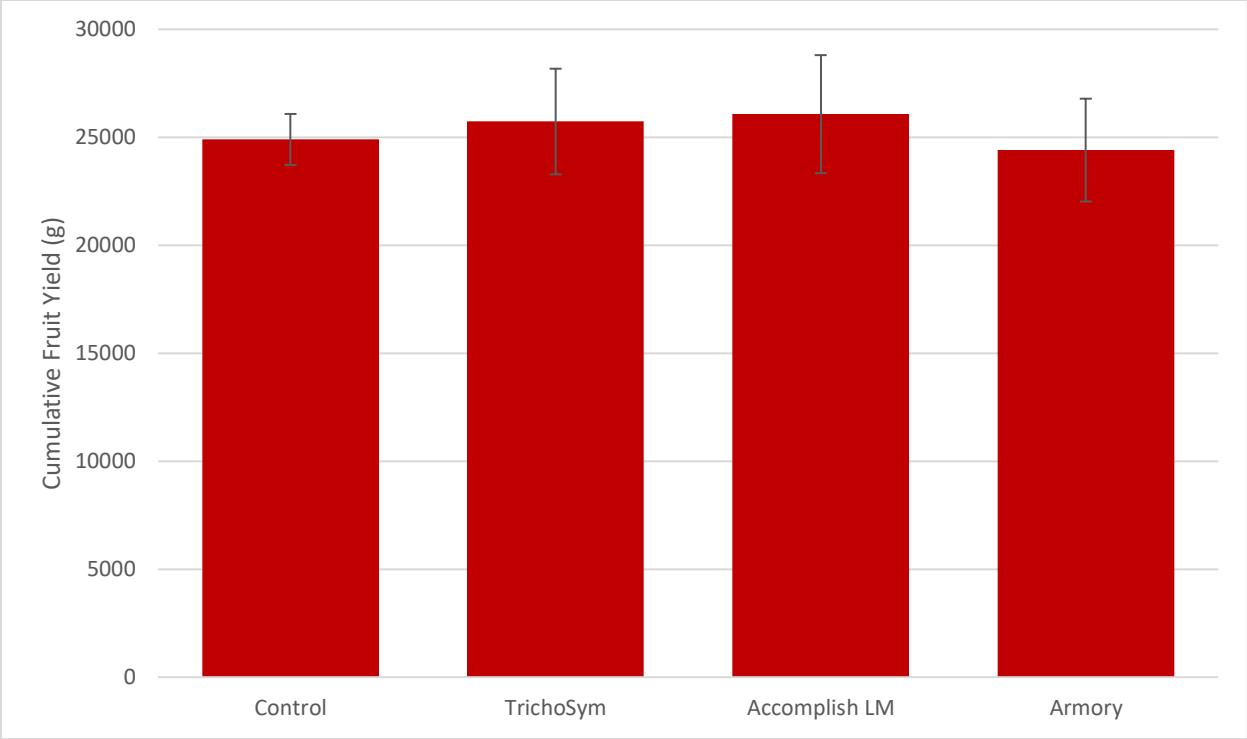


Figure 4-3: Seasonal cumulative Chandler strawberry fruit yield (g) by biostimulant treatment. Error bars represent one standard deviation of the mean.

The control plots (X; n = 4) had a combined cumulative seasonal fruit yield of 24900.0 ± 254.7 g; the plots treated with TrichoSym Bio (n = 4) had a combined cumulative seasonal fruit yield of 25731.6 ± 301.9 g; plots treated with Accomplish LM (n = 4) had a combined cumulative seasonal fruit yield of 26071.7 ± 295.9 g; and plots treated with Armory (n = 4) had a combined cumulative seasonal fruit yield of 24407.7 ± 281.7 grams (Fig. 4-3).

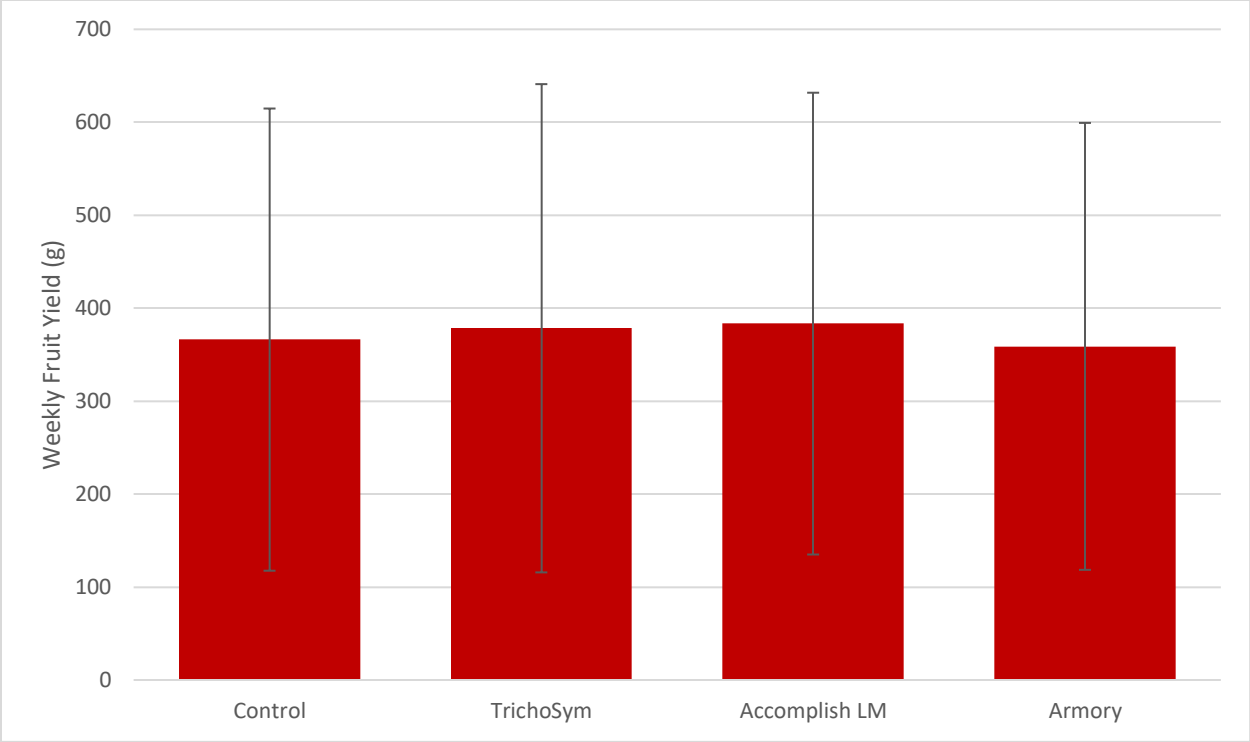


Figure 4-4: Weekly average Chandler strawberry fruit yield (g) by biostimulant treatment. Error bars represent one standard deviation of the mean.

Biostimulant product treatment did not have a significant effect (P = 0.912) on cumulative fruit yield over the course of the entire growing season (Fig. 4-3); nor did product treatment have a significant effect (P = 0.912) on weekly average fruit yield (Fig. 4-4).

After rating the stress/disease level of each individual plant on a categorical scale from 0 to 4, a disease severity percentage rating was calculated for each treatment, at each time point. The same disease severity percentages were calculated for each block, at the same time points. These disease severity percentages were then used to create graphs of disease progress over time, which are shown below for each treatment Fig. 4-5.

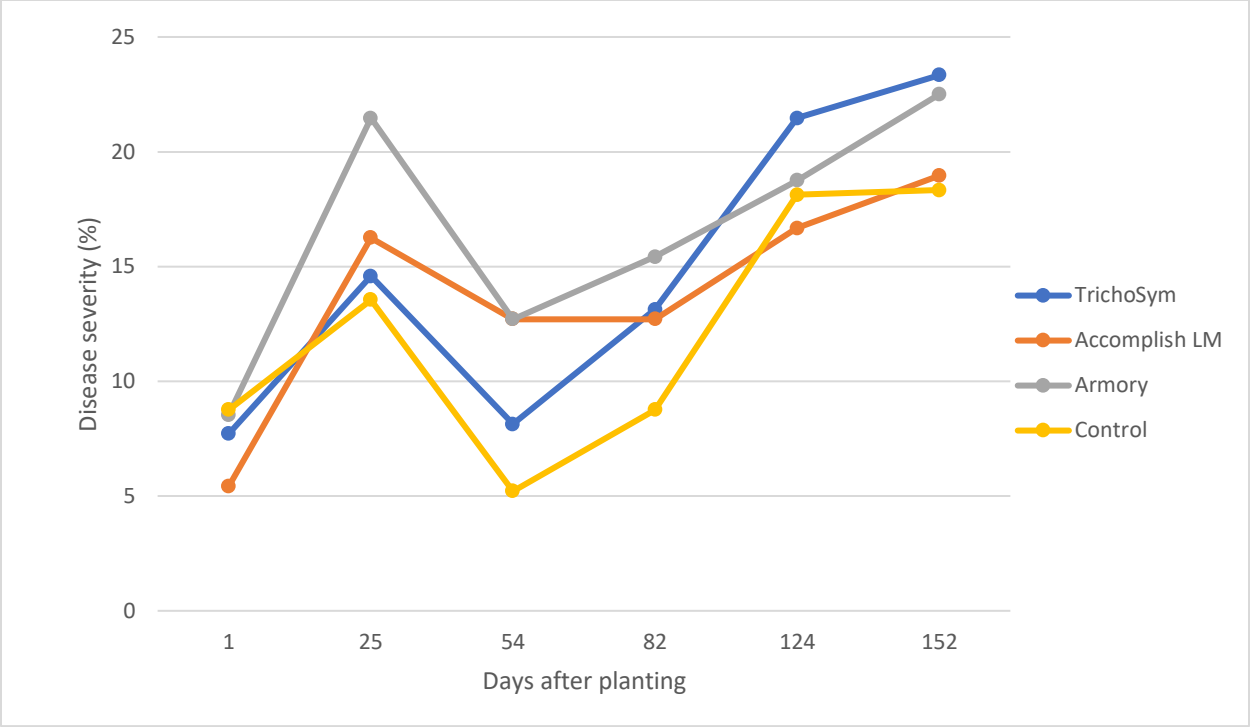


Figure 4-5: Disease progress over time for all treatments during 2020 growing season; 1/27/20 = day 1. Each data point represents an average of four points (n = 4).

The preliminary soil samples taken on January 27 (prior to any treatment application) from each of the 4 blocks all had *Verticillium* levels of less than 3 CFU/g (0.625 – 2.292 CFU/g), which is generally considered to be the threshold above which strawberry plants will become diseased. Neither block nor treatment had a significant effect on total CFU/g soil (P = 0.2154 and 0.5718, respectively). These numbers continued to go down as the season progressed, except block 4, which increased slightly but stayed under one CFU/g soil. This is likely because *V. dahliae* prefers temperatures between 21-27°C and temperatures increased to above this range later in the season (Berlanger and Powelson, 2000). By June 26, the soil samples from all four blocks averaged below 1 CFU/g soil, and again, neither block nor treatment affected the total CFU/g soil significantly (P = 0.3865 and 0.0899, respectively).

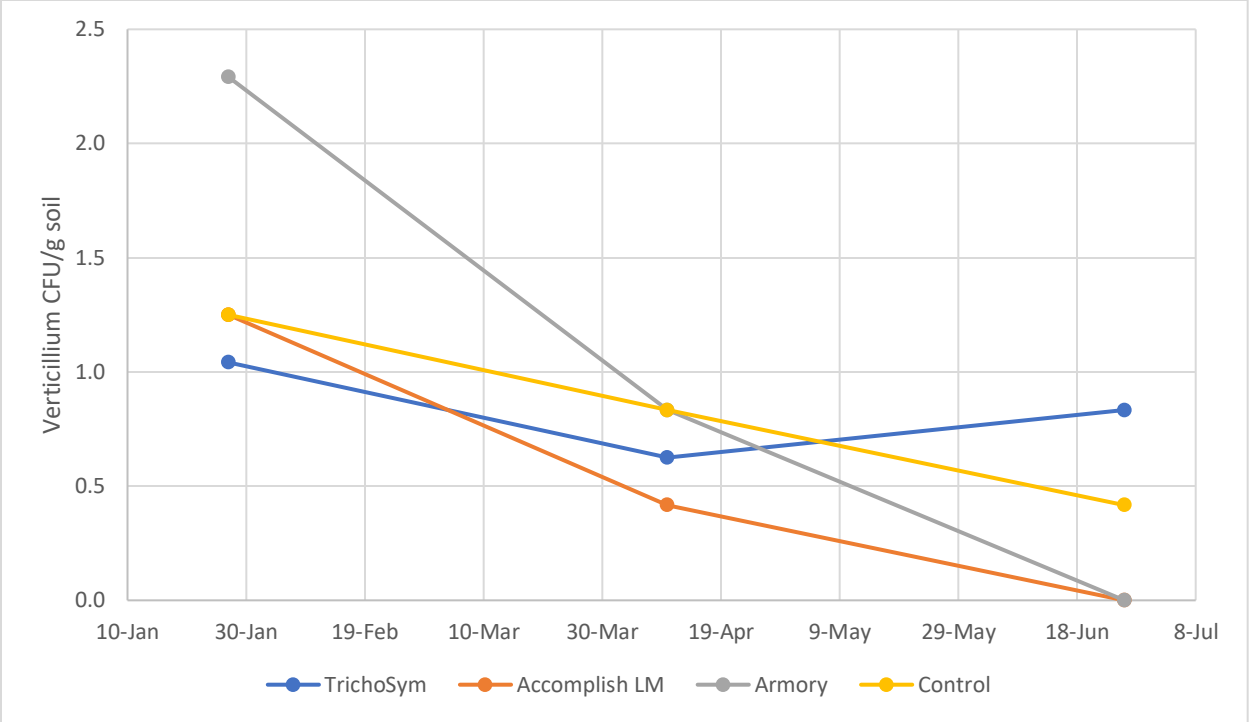


Figure 4-6: Soil *Verticillium* presence (in colony-forming units, or CFU) by biostimulant treatment. Different lines represent different treatments.

Figure 4-6 shows the soil samples that were analyzed for *Verticillium* presence, organized by treatment. All three of the treatments as well as the control had low numbers of CFU per gram initially (0.000-3.333 CFU/g), and the *Verticillium* numbers went down in all 3 treatments and the control from January 27 to April 10. Inoculum levels decreased over time from January to June, but this difference was not significant ($P = 0.359$). The samples taken on June 26 were all below 1 CFU/g soil, on average.

4.4 Discussion

Echoing the results of the hoophouse trial from the previous year, this field trial found that the microbial biostimulant products tested had no significant impacts on marketable fruit yield, soil *V. dahliae* presence, or disease severity as indicated by AUDPC. The significant differences in fruit yield between blocks observed in this trial were more likely due to naturally occurring land variability that was not apparent at the

outset of the study, or population differences of other soilborne pathogens that were not tested in this study, rather than differences in *V. dahliae* pathogen load, as tests on *V. dahliae* CFU/g soil showed no significant differences across blocks. Further research on PGPR and PGPF soil supplementation in strawberries should include a protocol for root-dipping the strawberry bare-root crowns in a liquid microbial suspension prior to planting, in addition to subsequent soil drench applications, as this would likely increase the likelihood of successful rhizosphere colonization by the beneficial microbes.

One previously mentioned study found that *B. subtilis* inoculation of bare-root crowns essentially neutralized the effects of the concurrent *V. dahliae* inoculation (Tahmatsidou, 2006). Two of the products tested in this study, EM-1 and Armory, also contained *B. subtilis*; however, in this study the PGPR inoculum was applied approximately two months after planting, rather than pre-planting, and the soil was not pre-fumigated with methyl bromide, as it was in the 2006 Tahmatsidou study (although those researchers suggested repeating their experiment in non-fumigated soil). Esitken et al. (2010) found that *Bacillus* root inoculation of strawberry plants significantly increased yield, which was not found in the trials described in this paper. A liquid supplement containing *Bacillus* along with *Azotobacter* and *Derxia* genera was also reported to have significantly increased not only fruit yield per plant but also fruit sugar content in strawberries in both years of a two-year study (Pesakovic et al., 2013). Soil supplementation with *T. harzianum* was found to increase strawberry yield relative to the untreated control over the course of a three-year study; this increase ranged widely from just 6.5% in the first year to 84.9% in the second year (Porras et al., 2007) .

A major pitfall in the experimental design of this study was that, as mentioned before, dead plants suspected of succumbing to *V. dahliae* infestation were not actually collected and plated to confirm that *V. dahliae* was in fact the cause of the plants' death, rather than an abiotic cause (e.g., lack of nutrient availability or water stress). This would have taken considerably more time and effort but would have been worthwhile. Any future trials with research questions similar to those in this study would certainly benefit from much more in-depth pathological analysis of individual diseased or dead plants. Also, future studies of a similar nature should include protocols to ensure that the microbial soil amendments are still viable throughout the duration of the study. Roots of treated plants could be dug up and tested for the presence of specific isolates they were inoculated with (such as *B. subtilis*) several times over the course of the study. Further discussion of the shortcomings of these trials, as well as future research directions, are contained in the following chapter.

CHAPTER 5

Conclusions

This study was comprised of two distinct trials aimed to assess the efficacy of several commercially available biostimulant products for increasing fruit yield and lessening disease severity in organically grown strawberry plants. The two trials were distinct in that the first trial used potted plants grown in a hoophouse and incorporated two different soil types, sandy and clay loam; the second trial was a field trial using only sandy soil.

According to our findings, none of the products tested managed to exert any significant effect on the growth, health, or yield of our strawberry plants in either of the two trials conducted. In both trials, every effort was undertaken to ensure that the products were applied properly, consistently and at the maximum frequency to optimize any potential results. Each application was a fairly time-consuming process, which may have been justified if significant positive results on either marketable fruit yield or stress tolerance had been measured. These trials cannot report any such results or other findings indicating that these liquid or powdered microbial soil amendments are genuinely beneficial to strawberry plants in a way that is substantial and reproducible in a field or semi-controlled environment setting.

Growers should be discerning when purchasing some of these products, as many of their claims can be somewhat dubious and unregulated, and the products may not have been tested extensively before putting them on the market. There is also the issue of the very high cost of many of these products, as well as the time and labor required to apply them. Perhaps microbial biostimulants of this nature would be more effective in

combination with other treatment or strategies, such as soil solarization or crop rotation with a non-host plant such as broccoli, both of which have shown promising results on their own (Njoroge et al., 2009).

This study had several limitations; most notably, the trials were not repeated over multiple growing seasons, which would have greatly increased the reliability of any findings, whether significant or not. In a field trial such as the one in this study, the quality of the research is inherently dependent on the naturally occurring levels of the pathogen(s) in the soil. In this case, the underlying levels of soilborne *V. dahliae* in the field where the study took place were already quite low, below the wilt threshold of approximately 3 CFU/g in strawberry plants. Performing extensive soil tests in order to ensure that the experimental field soil is already inoculated with relatively high levels of *V. dahliae* would ensure that any treatment effects from PGPR and/or PGPF application would be more noticeable and measurable. Also, having multiple experimental plots across several different fields, each with their own unique soil microbial composition, would have likely produced results with lower standard error, and perhaps may have even illuminated more potential benefits of applying these treatment products.

At the end of these trials, we cannot conclude that these particular microbial products definitively represent a promising alternative to the practice of soil fumigation with methyl bromide or other, similar fumigant chemicals in the strawberry industry. This study does not, however, imply that the product treatments tested herein, or other similar products on the market, would not work in strawberry fields with higher levels of soilborne pathogen activity, or for other crops with different physiologies.

Breeding for genetic resistance to *V. dahliae* and other soilborne pathogens is still one of the strawberry industry's best options, and nowadays, genetic engineering is also a promising option. Moving forward, research in strawberry cultivation should focus most on finding ways to minimize the impact of ozone-depleting fumigant chemicals while still making judicious usage of them, and especially on breeding to develop more disease-resistant strawberry cultivars.

References

Almaghrabi, O. A., Massoud, S. I., and Abdelmoneim, T. S. 2013. Influence of inoculation with plant growth promoting rhizobacteria (PGPR) on tomato plant growth and nematode reproduction under greenhouse conditions. *Saudi Journal of Biological Sciences*, *20*(1), 57–61.

Anter, H. 2003. Effects of biofertilization under varying NPK levels on growth, yield and fruit quality of strawberry plants. *J. Agric. & Env. Sci. Alex. Univ., Egypt*, 2, 106-129.

Antoun, H. 2013. Plant-Growth-Promoting Rhizobacteria. *Brenner's Encyclopedia of Genetics: Second Edition*, 353–355.

Berlanger, I. and M.L. Powelson. 2000. Verticillium wilt. The Plant Health Instructor. DOI: 10.1094/PHI-I-2000-0801-01. Accessed 18 Dec 2020.

Beneduzi, A., Ambrosini, A., & Passaglia, L. M. P. 2012. Plant growth-promoting rhizobacteria (PGPR): Their potential as antagonists and biocontrol agents. *Genetics and Molecular Biology*, 35(4), 1044–1051.

Blauer, K. and Holmes, G. 2019. Evaluation of pre-plant dip, biofumigant, and soil-drench treatments to control Verticillium wilt on fall-planted strawberries. Plant Disease Management Reports. Retrieved from https://content-calpoly-edu.s3.amazonaws.com/strawberry/1/documents.pdf. Accessed 26 Dec 2020.

Cheol Kim, Y., Jung, H., Yong Kim, K., and Kee Park, S. 2007. An effective biocontrol bioformulation against Phytophthora blight of pepper using growth mixtures of combined chitinolytic bacteria under different field conditions. *European Journal of Plant Pathology* 120, 372-383.

Collier, J. 2012. Growing strawberries. Retrieved from https://ucanr.edu/blogs/blogcore/postdetail.cfm?postnum=8792. Accessed 1 April 2021.

Cooper, J. 2007. Fungal Plant Pathogens and Symptomology. Retrieved from https://s3.wp.wsu.edu/uploads/sites/2054/2014/04/FungalPlantPathogens_002.pdf. Accessed 20 Aug 2019.

Du Jardin, P. 2015. Plant biostimulants: Definition, concept, main categories and regulation. *Scientia Horticulturae*, 196, 3–14.

Esitken, A., Yildiz, H. E., Ercisli, S., Figen Donmez, M., Turan, M., and Gunes, A. 2010. Effects of plant growth promoting bacteria (PGPB) on yield, growth and nutrient contents of organically grown strawberry. *Scientia Horticulturae*, 124(1), 62–66.

Fang, X., Phillips, D., Verheyen, G., Li, H., Sivasithamparam, K and Barbetti, A. 2012. Yield and resistance of strawberry cultivars to crown and root diseases in the field, and cultivar responses to pathogens under controlled environment conditions. *Phytopathologia Mediterranea* 51(1), 69-84.

Glick, Bernard. 2012. "Plant Growth-Promoting Bacteria: Mechanisms and Applications," Scientifica, Vol. 2012. Retrieved from https://doi.org/10.6064/2012/963401.

Gordon, T., Kirkpatrick, S., Hansen, J., and Shaw, D. 2006. Response of strawberry genotypes to inoculation with isolates of *Verticillium dahliae* differing in host origin. *Plant Path.* 55(6), 766-769.

Gül, A., Özaktan, H., Kidoğlu, F., and Tüzel, Y. 2013. Rhizobacteria promoted yield of cucumber plants grown in perlite under Fusarium wilt stress. *Scientia Horticulturae*, 153, 22–25.

Hayek, S., Grosch, R., Gianinazzi-Pearson, V., and Franken, P. 2012. Bioprotection and alternative fertilisation of petunia using mycorrhiza in a soilless production system. *Agron. Sustain. Dev.* 32, 765-771.

Holmes, G., Mansouripour, S., and Hewavitharana, S. 2020. Strawberries at the crossroads: management of soilborne diseases in California without methyl bromide. *Phytopathology* 110, 956-968.

Kabir, Z., Bhat, R., and Subbarao, K. 2004. Comparison of media for recovery of *Verticillium dahliae* from soil. *Plant Dis*. 88, 49-55.

Kang, S.-M., Gil-Jae, A. E., Ae, J., Hamayun, M., Na, C., Shin, D., Kim, H. Y., Hong, J. and I. Lee. 2008. Gibberellin production and phosphate solubilization by newly isolated strain of Acinetobacter calcoaceticus and its effect on plant growth. *Biotechnology Letters*, 31, 277-281.

Khan, M. S., Zaidi, A., & Musarrat, J. 2009. Microbial strategies for crop improvement. *Microbial Strategies for Crop Improvement*, (May 2014), 1–358.

Kunicki, E., Grabowska, A., Sękara, A., and Wojciechowska, R. 2010. The effect of cultivar type, time of cultivation, and biostimulant treatment on the yield of spinach (Spinacia oleracea L.). *Folia Horticulturae*, 22(2), 9–13.

Leandro, L., Guzman, T., Ferguson, L., Fernandez, G., and Louws, L. 2007. Population dynamics of *Trichoderma* in fumigated and compost-amended soil and on strawberry roots. *Applied Soil Ecology* 35(1), 237-246.

Lisiecka, J. 2011. The effect of animal protein hydrolysate on quantity and quality of strawberry daughter plants cv. 'Elsanta'. 2011. *Acta Sci. Pol., Hortorum Cultus* 10(1), 31-40.

Lloyd, M. and Gordon, T. 2012. Growing for the future: Collective action, land stewardship and soilborne pathogens in California strawberry production. *California Agriculture* 70(3), 101-103.

López-Bucio, J., Pelagio-Flores, R., and Herrera-Estrella, A. 2015. Trichoderma as biostimulant: exploiting the multilevel properties of a plant beneficial fungus. *Scientia Horticulturae*, 196, 109–123.

Lucini, L., Rouphael, Y., Cardarelli, M., Canaguier, R., Kumar, P., and Colla, G. 2015. The effect of a plant-derived biostimulant on metabolic profiling and crop performance of lettuce grown under saline conditions. *Scientia Horticulturae*, 182, 124–133.

Meadows, R. 2013. Researchers develop alternatives to methyl bromide fumigation. *California Agriculture*, 67(3), 125–127.

Nallanchakravarthula, S., Mahmood, S., Alstrom, S., and Finlay, R. 2014. Influence of soil type, cultivar, and *Verticillium dahliae* on the structure of the root and rhizosphere soil fungal microbiome of strawberry. PLoS ONE 9(10): e111455. Retrieved from https://doi.org/10.1371/journal.pone.0111455. Accessed 26 Dec 2020.

Njoroge, S., Kabir, Z., Martin, F., Koike, S. and Subbarao, K. 2009. Comparison of crop rotation for verticillium wilt management and effect on *Pythium* species in conventional and organic strawberry production. *Plant Dis.* 93, 519-527.

O'Sullivan, D. and F. O'Gara. 1992. Traits of fluorescent Pseudomonas spp. involved in suppression of plant root pathogens. *Microbiological Reviews* 56(4), 662-676.

Pešakovíc, M., Karaklajíc-Stajíc, Z., Milenkovíc, S., and O. Mitroví. 2013. Biofertilizer affecting yield related characteristics of strawberry (*Fragaria* × *ananassa* Duch.) and soil micro-organisms. *Scientia Horticulturae*, 150, 238–243.

Porras, M., Barrau, C., and Romero, F. 2007. Effects of soil solarization and Trichoderma on strawberry production. *Crop Protection*, 26, 782–787.

Reganold, J., Andrews, P., Reeve, J., Carpenter-Boggs, L., Schadt, C., Alldredge, J., Ross, C., Davies, N. and J. Zhou. 2010. Fruit and Soil quality of organic and conventional strawberry agroecosystems. PLoS ONE 5(9): e12346. Accessed 18 Dec 2020.

Rodríguez, H. and R. Fraga, 1999. "Phosphate solubilizing bacteria and their role in plant growth promotion," *Biotechnology Advances*, 17(5), 319–339.

Schulte, E. E. (n.d.). Understanding Plant Nutrients Soil and Applied Iron. Retrieved from http://corn.agronomy.wisc.edu/Management/pdfs/a3554.pdf. Accessed 18 Dec 2020.

Senthilraja, G., Anand, T., Kennedy, J. S., Raguchander, T., and R. Samiyappan. 2013. Plant growth promoting rhizobacteria (PGPR) and entomopathogenic fungus bioformulation enhance the expression of defense enzymes and pathogenesis-related proteins in groundnut plants against leafminer insect and collar rot pathogen. *Physiological and Molecular Plant Pathology* 82, 10-19.

Shaw, D. V, Gubler, W. D., Larson, K. D., Hansen, J., Gubler, W. D., Larson, K. D., and Hansen, J. 1996. Genetic Variation for Field Resistance to Verticillium dahliae Evaluated Using Genotypes and Segregating Progenies of California Strawberries. *J. Amer. Soc. Hort. Sci.*, 121(4), 625–628.

Smith, S. E., Jakobsen, I., Grønlund, M., and Smith, F. A. 2011. Roles of arbuscular mycorrhizas in plant phosphorus nutrition: interactions between pathways of phosphorus uptake in arbuscular mycorrhizal roots have important implications for understanding and manipulating plant phosphorus acquisition. *Plant Physiology*, 156(3), 1050–1057.

Spalding, R. F., and Exner, M. E. 1993. Occurrence of Nitrate in Groundwater-A Review. Retrieved from https://dl-sciencesocieties-org.ezproxy.lib.calpoly.edu/publications/jeq/pdfs/22/3/JEQ0220030392. Accessed 18 Dec 2020.

Tahmatsidou, V., O'Sullivan, J., Cassells, A., Voyiatzis, D., and Paroussi, G. 2006. Comparison of AMF and PGPR inoculants for the suppression of verticillium wilt of strawberry. *Applied Soil Ecology,* 32, 316-324.

Tarantino, A., Lops, F., Disciglio, G., and Lopriore, G. 2018. Effects of plant biostimulants on fruit set, growth, yield and fruit quality attributes of 'Orange rubis®' apricot (Prunus armeniaca L.) cultivar in two consecutive years. *Scientia Horticulturae*, 239, 26–34.

Tronsmo A., Hjeljord, L., and Stensvand, A. 1999. Effect of temperature and nutrient stress on the capacity of commercial *Trichoderma* products to control *Botrytis cinerea* and *Mucor piriformis* in greenhouse strawberries. *Biological Control,* 19(2), 149-160.

Tsavkelova, E.A., Klimova, S.Y., and Cherdyntseva, T.A. 2006. Microbial producers of plant growth stimulators and their practical use: a review. *Applied Biochemistry and Microbiology* (2006) 4, 117-126.

Tubeileh, A. and Stephenson, G. 2020. Soil amendment by composted plant wastes reduces the *Verticillium dahliae* abundance and changes soil chemical properties in a bell pepper cropping system. *Current Plant Biology,* 22(2020).

University of California, B. D. of A. and N. R. 1994. California agriculture. *California Agriculture* (Vol. 48). [California Agricultural Experiment Station]. Retrieved from http://calag.ucanr.edu/Archive/?article=ca.v048n03p7. Accessed 18 Dec 2020.

Vansuyt, G., Robin, A. Briat, J.F. Curie, C. and Lemanceau, P. 2007. "Iron acquisition from Fe-pyoverdine by Arabidopsis thaliana," *Molecular Plant-Microbe Interactions*, 20(4), 441–447.

Vessey, J. K. 2003. Plant growth promoting rhizobacteria as biofertilizers. *Plant and Soil*, 255(2), 571–586.

Xu, C., and Mou, B. 2017. Drench Application of Fish-derived Protein Hydrolysates Affects Lettuce Growth, Chlorophyll Content, and Gas Exchange. *HortTechnology*, 27(4), 539–543.

Zahran, H. 1999. Rhizobium-legume symbiosis and nitrogen fixation under severe conditions and in an arid climate. *Microbiol Mol Biol Rev*. 63(4), 968-89.

Zehnder, G. W., Yao, C., Murphy, J. F., Sikora, E. R., and Kloepper, J. W. 2000. Induction of resistance in tomato against cucumber mosaic cucumovirus by plant growth-promoting rhizobacteria. *BioControl*, 45(1), 127–137.

Appendix A

Layout of 2018-2019 potted plant experiment (160 plants total). Treatments in blue represent clay soil; treatments in orange represent sandy soil.

Block 1	Block 2	Block 3	Block 4	Block 5	Block 6	Block 7	Block 8	Block 9	Block 10
Acc. LM	EM-1	Acc. LM	Acc. LM	Armory	Armory	Armory	EM-1	EM-1	Armory
Control	Armory	Armory	Acc. LM	Armory	Acc. LM	Control	Acc. LM	Armory	Control
EM-1	Control	Acc. LM	Control	EM-1	EM-1	Armory	EM-1	Control	EM-1
Armory	Acc. LM	Control	EM-1	EM-1	Acc. LM	EM-1	Armory	Control	EM-1
EM-1	Armory	EM-1	EM-1	Acc. LM	EM-1	Control	Armory	Armory	Armory
Acc. LM	Acc. LM	EM-1	Armory	Acc. LM	Control	EM-1	Control	EM-1	Control
Armory	Control	Armory	Armory	Control	Control	Acc. LM	Control	Acc. LM	Acc. LM
Control	EM-1	Control	Control	Control	Armory	Acc. LM	Acc. LM	Acc. LM	Acc. LM
Block 11	**Block 12**	**Block 13**	**Block 14**	**Block 15**	**Block 16**	**Block 17**	**Block 18**	**Block 19**	**Block 20**
Acc. LM	Control	EM-1	EM-1	EM-1	EM-1	Acc. LM	Armory	EM-1	Armory
Control	Armory	Control	Armory	Control	Control	Control	Armory	Armory	Acc. LM
EM-1	Acc. LM	Control	Control	Armory	Acc. LM	Armory	EM-1	Control	EM-1
Armory	Control	Armory	Armory	Control	Acc. LM	Armory	Acc. LM	Acc. LM	Armory
EM-1	Acc. LM	Acc. LM	Acc. LM	EM-1	Armory	EM-1	Control	EM-1	Acc. LM
Control	EM-1	Armory	Control	Acc. LM	Armory	EM-1	Acc. LM	Control	EM-1
Acc. LM	EM-1	Acc. LM	Acc. LM	Acc. LM	Control	Acc. LM	Control	Armory	Control
Armory	Armory	EM-1	EM-1	Armory	EM-1	Control	EM-1	Acc. LM	Control

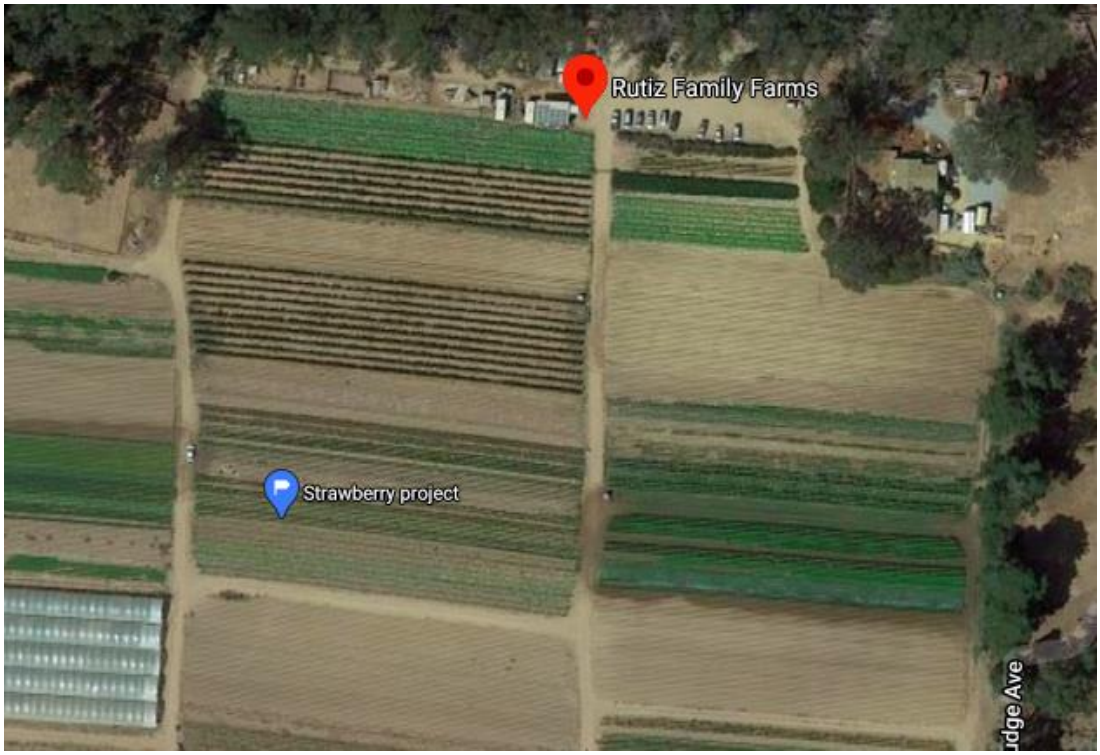
Plot layout of 2020 field experiment. White=control (X); Green=TrichoSym (A); Orange=Accomplish LM (B); Blue=Armory (C). Each row constituted one block (four blocks total), and each block contained four plots (16 plots total). Each plot contained 30 plants for a total of 480 plants.

Block 1 Accomplish LM	Block 1 TrichoSym	Block 1 Control	Block 1 Armory
Block 2 TrichoSym	Block 2 Accomplish LM	Block 2 Armory	Block 2 Control
Block 3 Armory	Block 3 Control	Block 3 TrichoSym	Block 3 Accomplish LM
Block 4 Control	Block 4 Armory	Block 4 Accomplish LM	Block 4 TrichoSym

Appendix B

Location of 2018-2019 strawberry biostimulant research project (shown in blue) within the Crops Unit, California Polytechnic State University, San Luis Obispo, California (Google Maps).

Location of 2020 strawberry biostimulant research project (shown in blue) within Rutiz Family Farms, Arroyo Grande, California. Rows are arranged in an east-west orientation (Google Maps).



Appendix C

Sorensen's NP-10 Medium, a selective medium for culturing *Verticillium dahliae*

(adapted from Kabir et al., 2004)

I) Add these ingredients to 2 separate flasks:

FLASK 1:

	500 mL medium	**1 L medium**
Distilled water	250 mL	500 mL
NaOH (1N)	12.5 mL	25 mL
Polygalacturonic acid (Na salt)	2.5 g	5.0 g

FLASK 2:

	500 mL medium	**1 L medium**
Distilled water	250 mL	500 mL
Agar	7.5 g	15.0 g
Potassium nitrate (KNO_3)	0.5 g	1.0 g
Potassium PO_4 monobasic (KH_2PO_4)	0.5 g	1.0 g
Potassium chloride (KCl)	0.25 g	0.5 g
Magnesium sulfate ($MgSO_4$-$7H_2O$)	0.25 g	0.5 g

Add a **stir bar** to flask 2.

II) Autoclave flasks 1 and 2 for 20 minutes.

III) Cool in water bath to 55°C.

IV) To flask 2, add antibiotic stock solutions (see below).

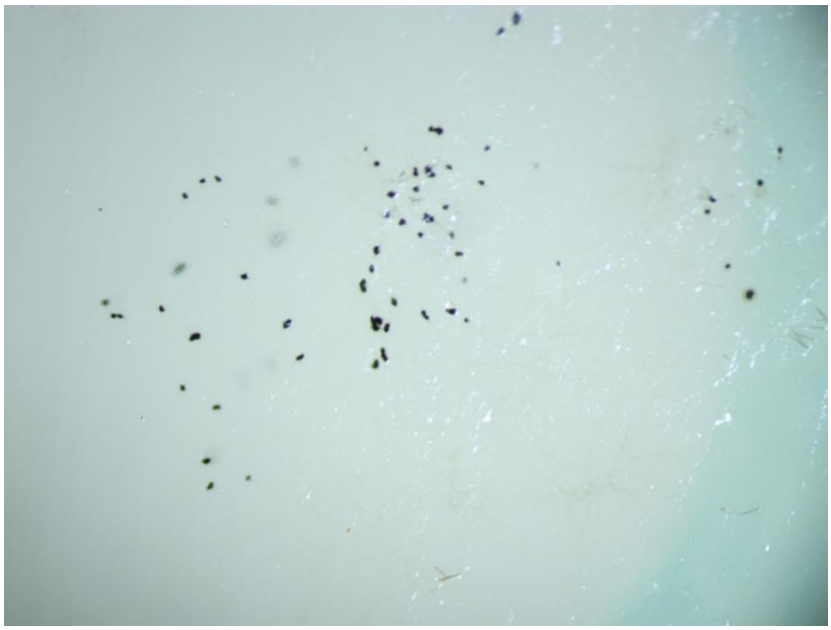
	500 mL medium	**1 L medium**

Streptomycin SO_4	25 mg	50 mg
Chlortetracycline HCl	25 mg	50 mg
Chloramphenicol	25 mg	50 mg
Tergitol NP-10 (filter sterilized)	0.25 mL (250 uL)	0.5 mL (500 uL)

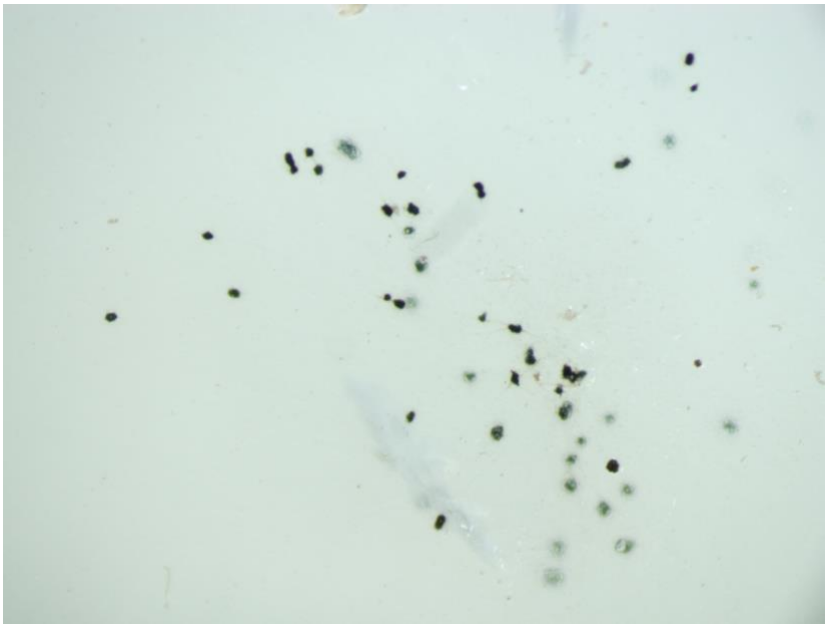
V) Pour flask 2 into flask 1, stir, and pour plates immediately.

Appendix D

Photos of *Verticillium dahliae* colonies taken from soil samples plated on NP-10 media.



Photos of *Verticillium dahliae* colonies taken from soil samples plated on NP-10 media.



Appendix E

A & L Western Agricultural Laboratories soil analysis results.

A & L WESTERN AGRICULTURAL LABORATORIES

1311 WOODLAND AVE #1 • MODESTO, CALIFORNIA 95351 • (209) 529-4080 • FAX (209) 529-4736

REPORT NUMBER: 20-265-051 CLIENT NO. 9999-D SUBMITTED BY: MARY MAHER

SEND TO: DR. ASHRAF TUBEILEH GROWER: JERRY RUTZ

DATE OF REPORT: 09/23/20

SOIL ANALYSIS REPORT

PAGE 1

SAMPLE ID	LAB NUMBER	Organic Matter		Phosphorus		Potassium	Magnesium	Calcium	Sodium	pH		Hydrogen	Cation Exchange Capacity	PERCENT CATION SATURATION (COMPUTED)				
		% Rating	ENR lbs/A	PI (Weak Bray) ppm	NaHCO3-P (Olsen Method) ppm	K ppm	Mg ppm	Ca ppm	Na ppm	Soil pH	Buffer Index	H meq/100g	CEC meq/100g	K %	Mg %	Ca %	H %	Na %
1A000	57802	1.2L	54	128VH	40VH	43	146	676	40	7.3		0.0	4.9	2.3	24.7	69.4	0.0	3.6
2A000	57803	1.1L	53	130VH	49VH	54M	146H	722H	32L	7.4		0.0	5.1	2.7	23.6	71.0	0.0	2.7
3A000	57804	1.1L	52	132VH	46VH	44M	137H	725H	32L	7.4		0.0	5.0	2.3	22.6	72.4	0.0	2.8
4A000	57805	1.0L	51	129 *	54VH	47M	152H	749H	43M	7.6		0.0	5.3	2.3	23.6	70.6	0.0	3.5
1B000	57806	1.2L	55	134VH	49VH	61M	140H	752H	31L	7.5		0.0	5.2	3.0	22.2	72.2	0.0	2.6

* Weak Bray unreliable at M or H excess lime or pH > 7.5

SAMPLE NUMBER	Nitrogen NO_3-N	Sulfur SO_4-S	Zinc Zn	Manganese Mn	Iron Fe	Copper Cu	Boron B	Excess Lime Rating	Soluble Salts mmhos/cm	Chloride Cl	PARTICLE SIZE ANALYSIS SAND %	SILT %	CLAY %	SOIL TEXTURE
	ppm	ppm	ppm	ppm	ppm	ppm	ppm			ppm				
1A000	4.4VH	39VH	3.9H	4M	8L	0.5L	0.2VL	L	1.3M					
2A000	24M	28H	3.4H	4M	7L	0.5L	0.2VL	L	1.2M					
3A000	25M	30H	3.8H	4M	7L	0.6L	0.2VL	L	1.2M					
4A000	33H	38VH	3.9H	3M	7L	0.6L	0.2VL	L	1.5M					
1B000	24M	34H	3.7H	4M	7L	0.6L	0.2VL	L	1.4M					

* CODE TO RATING: VERY LOW (VL), LOW (L), MEDIUM (M), HIGH (H), AND VERY HIGH (VH).
-- ENR - ESTIMATED NITROGEN RELEASE
— MULTIPLY THE RESULTS IN ppm BY 2 TO CONVERT TO LBS. PER ACRE OF THE ELEMENTAL FORM
— MULTIPLY THE RESULTS IN ppm BY 4.6 TO CONVERT TO LBS PER ACRE P2O5
— MULTIPLY THE RESULTS IN ppm BY 2.4 TO CONVERT TO LBS PER ACRE K2O
MOST SOILS WEIGH TWO (2) MILLION POUNDS (DRY WEIGHT) FOR AN ACRE OF SOIL 6-28 INCHES DEEP

This report applies only to the sample(s) tested. Samples are retained a maximum of thirty days after testing.

Rogel Rogers, CCA, PCA
A & L WESTERN LABORATORIES, INC.

A & L WESTERN AGRICULTURAL LABORATORIES

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REPORT NUMBER: 20-265-051

CLIENT NO: 9999-D

SEND TO: DR. ASHRAF TUBEILEH

SUBMITTED BY: MARY MAHER

GROWER: JERRY RUTIZ

DATE OF REPORT: 09/23/20

SOIL ANALYSIS REPORT

SAMPLE ID	LAB NUMBER	Organic Matter		Phosphorus		Potassium	Magnesium	Calcium	Sodium	pH		Hydrogen	Cation Exchange Capacity	PERCENT CATION SATURATION (COMPUTED)				
		* % Rating	** ENR lbs/A	Pl (Weak Bray) *** ppm	$NaHCO_3$-P (Olsen Method) *** ppm	K ***** ppm	Mg *** ppm	Ca *** ppm	Na *** ppm	Soil pH	Buffer Index	H meq/100g	C.E.C. meq/100g	K %	Mg %	Ca %	H %	Na %
29000	57607	1.1L	52	133VH	46VH	48M	148H	718H	35L	7.4		0.0	5.1	2.4	23.9	70.7	0.0	3.0
39000	57608	1.1L	53	130VH	49VH	52M	144H	720H	31L	7.5		0.0	5.0	2.6	23.4	71.2	0.0	2.7
49000	57609	1.2L	54	133VH	48VH	70M	159H	778M	46M	7.4		0.0	5.6	3.2	23.4	69.8	0.0	3.6
1C000	57610	0.9L	47	129VH	48VH	48	138	682	35	7.5		0.0	4.8	2.6	23.6	70.7	0.0	3.1
2C000	57611	0.9L	48	130VH	53VH	51M	148H	724H	36M	7.4		0.0	5.1	2.6	23.7	70.6	0.0	3.1

SAMPLE NUMBER	Nitrogen NO_3-N ppm	Sulfur SO_4-S ppm	Zinc Zn ppm	Manganese Mn ppm	Iron Fe ppm	Copper Cu ppm	Boron B ppm	Excess Lime Rating	Soluble Salts mmhos/cm	Chloride Cl ppm		PARTICLE SIZE ANALYSIS			
												SAND %	SILT %	CLAY %	SOIL TEXTURE
29000	29H	28H	3.5H	4M	7L	0.5L	0.2VL	L	1.3M						
39000	23M	28H	3.7H	3M	8L	0.9M	0.2VL	L	1.2M						
49000	44VH	45VH	3.8H	3M	7L	0.6L	0.2VL	L	1.7M						
1C000	16M	35H	4.0H	3M	7L	0.6L	0.2VL	L	1.2M						
2C000	21M	39VH	4.0H	4M	9L	0.6L	0.2VL	L	1.3M						

* CODE TO RATING: VERY LOW (VL), LOW (L), MEDIUM (M), HIGH (H), AND VERY HIGH (VH).

** ENR - ESTIMATED NITROGEN RELEASE

*** MULTIPLY THE RESULTS IN ppm BY 2 TO CONVERT TO LBS. PER ACRE OF THE ELEMENTAL FORM

**** MULTIPLY THE RESULTS IN ppm BY 4.6 TO CONVERT TO LBS. PER ACRE P_2O_5

***** MULTIPLY THE RESULTS IN ppm BY 2.4 TO CONVERT TO LBS. PER ACRE K_2O

MOST SOILS WEIGH TWO (2) MILLION POUNDS (DRY WEIGHT) FOR AN ACRE OF SOIL 0-28 INCHES DEEP

This report applies only to the sample(s) tested. Samples are retained a maximum of thirty days after testing.

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REPORT NUMBER: 20-265-051

CLIENT NO: 9999-D

SEND TO: DR. ASHRAF TUBEILEH

SUBMITTED BY: MARY MAHER

GROWER: JERRY RUTIZ

DATE OF REPORT: 09/23/20

SOIL ANALYSIS REPORT

SAMPLE ID	LAB NUMBER	Organic Matter		Phosphorus		Potassium	Magnesium	Calcium	Sodium	pH		Hydrogen	Cation Exchange Capacity	PERCENT CATION SATURATION (COMPUTED)				
		*	**	Pt (Weak Bray)*	$NaHCO_3$-P (Olsen Method)	K	Mg	Ca	Na	Soil	Buffer	H	CEC	K	Mg	Ca	H	Na
		% Rating	ENR lbs/A	ppm	ppm	ppm	ppm	ppm	ppm	pH	Index	meq/100g	meq/100g	%	%	%	%	%
3C000	57612	1.1L	51	129 *	48VH	54M	142H	712H	36M	7.6		0.0	5.0	2.7	23.3	70.8	0.0	3.1
4C000	57613	0.9L	49	126 *	48VH	51M	158H	757M	46M	7.6		0.0	5.4	2.4	24.0	69.8	0.0	3.7
1X000	57614	0.9L	48	130VH	62VH	51M	137H	713H	37M	7.4		0.0	5.0	2.6	22.6	71.5	0.0	3.2
2X000	57615	0.9L	47	130 *	48VH	50M	140H	723H	34L	7.6		0.0	5.0	2.5	22.8	71.7	0.0	3.0
3X000	57616	1.0L	49	132VH	46VH	52M	160H	754M	40M	7.4		0.0	5.4	2.5	24.4	69.8	0.0	3.3

* Weak Bray unreliable at M or H excess lime or pH > 7.5

SAMPLE NUMBER	Nitrogen NO_3-N	Sulfur SO_4-S	Zinc Zn	Manganese Mn	Iron Fe	Copper Cu	Boron B	Excess Lime	Soluble Salts	Chloride Cl		PARTICLE SIZE ANALYSIS			
	ppm	ppm	ppm	ppm	ppm	ppm	ppm	Rating	mmhos/cm	ppm		SAND %	SILT %	CLAY %	SOIL TEXTURE
3C000	16M	42VH	3.3H	3M	6L	0.6L	0.2VL	L	1.3M						
4C000	28H	48VH	4.0H	3M	7L	0.6L	0.2VL	L	1.7M						
1X000	30H	35H	3.7H	4M	6L	0.5L	0.2VL	L	1.4M						
2X000	18M	33H	3.6H	3M	6L	0.6L	0.2VL	L	1.0M						
3X000	38H	43VH	3.9H	4M	6L	0.6L	0.2VL	L	1.5M						

* CODE TO RATING: VERY LOW (VL), LOW (L), MEDIUM (M), HIGH (H), AND VERY HIGH (VH).

** ENR - ESTIMATED NITROGEN RELEASE

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SEND TO: DR. ASHRAF TUBEILEH

SUBMITTED BY: MARY MAHER

GROWER: JERRY RUTIZ

DATE OF REPORT: 09/23/20

SOIL ANALYSIS REPORT

SAMPLE ID	LAB NUMBER	Organic Matter		Phosphorus		Potassium	Magnesium	Calcium	Sodium	pH		Hydrogen	Cation Exchange Capacity	PERCENT CATION SATURATION (COMPUTED)				
		*	**	P1 (Weak Bray)	$NaHCO_3$-P (Olsen Method)	K	Mg	Ca	Na	Soil	Buffer	H	C.E.C.	K	Mg	Ca	H	Na
		% Rating	ENR lbs/A	ppm	ppm	ppm	ppm	ppm	ppm	pH	Index	meq/100g	meq/100g	%	%	%	%	%
40000	57617	1.0L	49	129 *	47VH	63M	142H	707H	32L	7.6		0.0	5.0	3.2	23.3	70.6	0.0	2.8

* Weak Bray unreliable at M or H excess lime or pH > 7.5

SAMPLE NUMBER	Nitrogen NO_3-N	Sulfur SO_4-S	Zinc	Manganese	Iron	Copper	Boron	Excess Lime	Soluble Salts	Chloride		PARTICLE SIZE ANALYSIS			
												SAND	SILT	CLAY	SOIL TEXTURE
	ppm	ppm	ppm	ppm	ppm	ppm	ppm	Rating	mmhos/cm	ppm		%	%	%	
40000	15M	23M	3.6H	3M	8L	0.6L	0.2VL	L	1.1M						

* CODE TO RATING: VERY LOW (VL), LOW (L), MEDIUM (M), HIGH (H), AND VERY HIGH (VH)

** ENR - ESTIMATED NITROGEN RELEASE

— MULTIPLY THE RESULTS IN ppm BY 2 TO CONVERT TO LBS. PER ACRE OF THE ELEMENTAL FORM

— MULTIPLY THE RESULTS IN ppm BY 4.6 TO CONVERT TO LBS. PER ACRE P_2O_5

— MULTIPLY THE RESULTS IN ppm BY 2.4 TO CONVERT TO LBS. PER ACRE K_2O

MOST SOILS WEIGH TWO (2) MILLION POUNDS (DRY WEIGHT) FOR AN ACRE OF SOIL 0-23 INCHES DEEP

This report applies only to the sample(s) tested. Samples are retained a maximum of thirty days after testing.

Rogell Rogers, CCA, PCA
A & L WESTERN LABORATORIES, INC.

Appendix F

Monthly average climate data for San Luis Obispo, California from October 2018 – September 2020. Obtained from the California Irrigation Management Information System (CIMIS).

San Luis Obispo - Central Coast Valleys - Station 52

Month Year	Total ETo (in)	Total Precip (in)	Avg Sol Rad (Ly/day)	Avg Vap Pres (mBars)	Avg Max Air Temp (°F)	Avg Min Air Temp (°F)	Avg Air Temp (°F)	Avg Max Rel Hum (%)	Avg Min Rel Hum (%)	Avg Rel Hum (%)	Avg Dew Point (°F)	Avg Wind Speed (mph)	Avg Soil Temp (°F)
Oct 2018	4.00	0.64 K	368	12.3	78.2	51.9	63.1	88	39	63	49.6	3.3 K	66.1
Nov 2018	2.96	4.55	278 K	9.5 K	74.0	48.5 K	60.1 K	81	36	56 K	41.2 K	3.4	60.9
Dec 2018	2.35	1.07	227	9.4	63.8	46.1	54.5	84	46	65	42.4	4.5 K	56.8 K
Tots/Avgs	9.31	6.3	291	10.4	72.0	48.8	59.2	84	40	61	44.4	3.7	61.3

San Luis Obispo - Central Coast Valleys - Station 52

Month Year	Total ETo (in)	Total Precip (in)	Avg Sol Rad (Ly/day)	Avg Vap Pres (mBars)	Avg Max Air Temp (°F)	Avg Min Air Temp (°F)	Avg Air Temp (°F)	Avg Max Rel Hum (%)	Avg Min Rel Hum (%)	Avg Rel Hum (%)	Avg Dew Point (°F)	Avg Wind Speed (mph)	Avg Soil Temp (°F)
Jan 2019	1.95	6.91	216	10.4	64.0	45.7	54.4	88	51	72	44.5	3.5	55.2
Feb 2019	1.98	7.48 K	284	8.9	57.9	40.0	48.8	92	53	74	40.7	3.4	53.3
Mar 2019	3.59	6.17 K	403 K	10.3	64.8	45.7	54.6	90	52	72	44.9	3.4	56.9
Apr 2019	4.53	0.19	500	12.2 K	69.0	49.1	58.1	93	56	74 K	49.7 K	3.3	62.2
May 2019	4.62	1.75	523	12.4	66.6	49.9	57.1	93	59	78	50.1	3.2	64.5
Jun 2019	5.67 K	0.00	614 K	14.0 K	73.5 K	54.2 K	62.0 K	92 K	56 K	75 K	53.4 K	3.1	68.8
Jul 2019	6.50 K	0.00	657	14.3	77.0 K	53.4	62.6 K	94 K	52 K	74 K	54.1 K	3.2	70.2
Aug 2019	6.03	0.00	591	15.2	79.7	55.5	64.9 K	93	49	73 K	55.8 K	3.1	71.3
Sep 2019	5.27 K	0.02	505	13.5	82.0 K	55.4 K	67.3 K	86 K	40 K	62 L	52.4 L	3.2	70.8
Oct 2019	4.71 K	0.01	419 K	8.7 K	80.0 K	50.8 L	62.5 K	72 K	24 K	47 L	40.5 L	3.5 K	64.8
Nov 2019	2.65	2.24	279	9.6 K	70.3	45.1	55.9	83	41	65 K	42.6 K	3.1 K	60.0
Dec 2019	1.66	3.91	185	10.3 K	62.5	45.2	53.3	91	53	74 K	44.8 K	3.1	56.2
Tots/Avgs	49.16	28.7	431	11.7	70.6	49.2	58.5	89	49	70	47.8	3.3	62.9

San Luis Obispo - Central Coast Valleys - Station 52

Month Year	Total ETo (in)	Total Precip (in)	Avg Sol Rad (Ly/day)	Avg Vap Pres (mBars)	Avg Max Air Temp (°F)	Avg Min Air Temp (°F)	Avg Air Temp (°F)	Avg Max Rel Hum (%)	Avg Min Rel Hum (%)	Avg Rel Hum (%)	Avg Dew Point (°F)	Avg Wind Speed (mph)	Avg Soil Temp (°F)
Jan 2020	2.44	0.43	256	9.3	63.9	45.1	53.9	84	47	66	42.3	4.4 K	54.0
Feb 2020	3.36	0.02	373	8.3	69.5	43.1 K	54.8 K	83	33	57 K	38.8 K	3.5	55.3
Mar 2020	3.07	5.75	358 K	10.2	62.9	45.2	53.0	91	53	74	44.7	3.2	57.9
Apr 2020	4.63	2.52 K	493	11.4	68.4	48.2 K	57.9 K	89	53	71 K	47.8 K	3.8	61.1
May 2020	6.24 K	0.01 K	627	12.1	74.4 K	50.8 K	61.9 K	89 K	45 K	65 K	49.4 K	3.7	66.5
Jun 2020	6.32 K	0.06	654	13.0 K	75.9 K	53.5 L	63.4 K	88 K	47 K	67 K	51.3 K	3.5	68.9
Jul 2020	6.27	0.00	634	13.9	75.4	53.8	62.4	91	52	73	53.3	3.3	70.4
Aug 2020	5.87 K	0.01	539	9.5 K	83.2 L	56.7 L	65.4 L	85 L	44 L	69 L	54.8 L	3.1	72.0 K
Sep 2020	4.52 K	0.01	436	10.9 K	81.0 L	53.2 L	64.1 L	93 L	45 L	70 L	53.5 L	3.0 K	69.6
Tots/Avgs	42.72	8.8	486	11.0	72.7	50.0	59.6	88	47	68	48.4	3.5	64.0