EVALUATING THE EFFECTS OF FOLIAR AND SYSTEMIC AERATED AQUEOUS VERMICOMPOST SOLUTIONS ON PLANT GROWTH AND PEST DENSITIES OF CITRUS NURSERY TREES

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SIGNATURE PAGE

THESIS:	EVAULATING THE EFFECTS OF FOLIAR AND SYSTEMIC AERATED AQUEOUS VERMICOMPOST SOLUTIONS ON PLANT GROWTH AND PEST DENSITIES OF CITRUS NURSERY TREES
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ABSTRACT

The California citrus industry is a significant aspect of the state economy and it is faced with numerous challenges, including ever-stricter regulations on the usage of synthetic pesticides and fertilizers, as well as the increasingly prevalent threat of the fatal citrus disease Huanglongbing, also known as HLB. In order to address these challenges, more environmentally benign methods for ensuring the productivity of citrus trees without increasing pesticide and synthetic fertilizer usage is an approach that needs to be explored. Aqueous aerated vermicompost solutions have shown promise in related scientific research, yet little research has been performed on citrus trees. An experiment was designed to evaluate the effects of a commercially available vermicompost product on vegetative growth and pest densities of thrips, Asian citrus psyllids, and leafminers on potted navel orange nursery trees. The four treatments were soil drench, foliar spray, combined soil drench with foliar spray, and a control. The biomass parameters to be measured were dry leaf biomass, dry shoot biomass, dry root biomass, and trunk diameter. Results for biomass were statistically insignificant, but overall biological trends were noted, with highest biomass and trunk diameter in the foliar spray treatment group, followed by soil drench group, soil drench combined with foliar spray group, and control group, respectively. Pest densities for ACP and thrips were also insignificant. However, Leafminer data suggested an overall statistically significant reduction in leafminer populations in all three treatment groups compared to the control group. These results indicate that it would be beneficial to study the effects of vermicompost treatments on citrus in greater depth.

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The Citrus Industry in California

Citrus cultivation has been a major aspect of the history of the development of California, which has been one of the most well-known areas suited for growing high quality fresh citrus (Ferguson & Grafton-Cardwell, 2014, p.5). Citrus cultivation in California began in the early part of the 19th century in the Los Angeles area and gradually expanded throughout the region, especially with the introduction of the seedless Washington navel orange (Ferguson & Grafton-Cardwell, 2014, p.5). Income produced from citrus sales both domestically and internationally brought in enough money to substantially fuel economic growth and infrastructure improvements that helped make California what it is today (Ferguson & Grafton-Cardwell, 2014, p.5). California's unique Mediterranean climate has the perfect blend of characteristics that are essential to growing citrus fruits of superior quality, most notably that it reaches a sufficiently low enough temperature for the fruit to ripen and develop a high enough sugar content, yet still maintains high enough temperatures for the fruit not to freeze under most circumstances and with proper frost protection measures (Ferguson & Grafton-Cardwell, 2014, p.5). Thus, citrus is an integral part of California's economy, history, and culture.

Currently, according to USDA statistical information, the total revenue of the California citrus industry totaled at approximately $2.1 billion in 2019 (USDA, 2019a) and produces roughly 88% of the fresh citrus produced in the entire United States (USDA, 2019c), contributing to 51% of the overall utilized citrus production in the nation (USDA, 2019b).

Citrus Pests, Asian Citrus Psyllids, and Huanglongbing

The most important topic with regard to pest control in the citrus industry is the increasing prevalence of the Asian citrus psyllid (ACP), which poses a substantial threat to citrus production in California (Grafton-Cardwell, Stelinski, & Stansly, 2013). ACP is the only known vector of Huanglongbing disease (HLB), which is a bacterium that affects the phloem of the plant by inhibiting nutrient transport (Grafton-Cardwell, Stelinski, & Stansly, 2013). It is difficult to detect until symptoms arise, which include mottling of leaves and yellowing of one sector of the canopy, as well as lopsided and bitter fruit, which results in an unmarketable product and thus renders the tree economically unviable (Grafton-Cardwell, Stelinski, & Stansly, 2013). Once the disease is transmitted, the tree slowly begins to die; there is no known cure for the disease to date (Grafton-Cardwell, Stelinski, & Stansly, 2013). Since ACP primarily feeds on new flush growth (Dreistadt, & University of California Integrated Pest Management Program, 2012, p. 166), all citrus trees are vulnerable to ACP damage and possible HLB transmission. Currently, the California citrus industry finds itself in dire circumstances and is struggling to avoid the catastrophic damage that the Florida citrus industry suffered after the spread of HLB (Spreen, Baldwin, & Futch, 2014).

In addition to ACP, there are also a number of other pests such as thrips, various mites, leafminers, and mealybugs, to name a few, which can cause problems with respect to structural damage to citrus trees and compromise fruit quality in producing citrus orchards, thereby reducing profitability (Dreistadt, S., & University of California Integrated Pest Management Program, 2012).

Anticipating Future Challenges in Citriculture

The potential economic impact of HLB on the California citrus industry is worthy of sincere consideration. The decimation of Florida's citrus industry after the spread of HLB has been widely documented and analyzed. According to information made available by the USDA, between 2008 and the present, Florida's overall utilized citrus production dropped roughly 62% (USDA, 2019b). In a 10-year period Florida was dethroned as the nation's leading citrus producer, producing roughly 71% of national citrus yields in 2008 compared to the most recent data that indicate that they now produce only 44% of utilized citrus production in the United States (USDA, 2019b). Considering the magnitude of the California citrus industry, a comparable 62% drop in production like that experienced in Florida would be a substantial blow to the state's economy. To fully appreciate the magnitude of the potential effects, it is important to consider the upstream and downstream economic impacts of the citrus industry. According to Bruce Babcock, Professor of Public Policy at the University of California, Riverside, the total economic impact of citrus production on the California economy in the 2016-2017 production season was approximately $7.1 billion, including upstream and downstream economic impacts (Babcock, 2018).

HLB has currently been detected in several areas in southern California, including Los Angeles County, Orange County, and Riverside County, with a more recent find in San Bernardino County (CDFA, 2019a). Although the areas in which HLB has been detected are still limited, ACP is present in California throughout well over half of the state, which creates a massive potential for HLB to take hold because it can easily spread throughout the ACP populations that are already present (CDFA, 2019a). Quarantines

have been established in the areas in which HLB has been detected (CDFA, 2019a). Thus far, the quarantines, in combination with coordinated spraying in commercial citrus groves and biocontrol efforts in residential areas, appear to have slowed the spread of HLB and it has still not been found in commercial groves in the San Joaquin Valley (CDFA, 2019b).

Because of the difficulty in detecting the disease, it is difficult to track the spread of HLB (Grafton-Cardwell, Stelinski, & Stansly, 2013). Many citrus trees can be asymptomatic for several years and are able to be a hub for the disease throughout that time period; meaning that ACP can feed on the tree and become infected, thereby spreading the disease to other trees before symptoms can even be noticed (Grafton-Cardwell, Stelinski, & Stansly, 2013). This creates a high level of urgency because of the possibility that the disease could already be present in areas that are commercially productive (Grafton-Cardwell, Stelinski, & Stansly, 2013).

At this point, the widely accepted approach to combat ACP and other pests lies in applying both foliar and systemic pesticides to kill off and repel infestations (Grafton-Cardwell, Stelinski, & Stansly, 2013). This is problematic as a long term solution due to the fact that pesticides not only can be harmful to humans under certain circumstances if proper protocols are not followed, but also compromise beneficial insects such as honeybees, natural predators, as well as microorganisms that are part of the natural defense system of the plant, and also contribute to increasing pest resistance (Dreistadt, S., & University of California Integrated Pest Management Program, 2012, p. 33-35). Hence, more and more pesticides have to be applied due to diminishing effectiveness, which is both economically and environmentally unsustainable (Grafton-Cardwell,

Stelinski, & Stansly, 2013). Thus, it is important to discover new solutions to pest management and plant pathogen resistance that are either conducive to, or at least harmless towards, beneficial insect and microbial populations (Grafton-Cardwell, Stelinski, & Stansly, 2013).

Another very important issue to consider is ensuring the ongoing productivity of citrus trees, especially if they become infected with HLB. In Florida, the primary method of ensuring that citrus groves remain productive is a strategy of removing diseased trees as soon as symptoms are visible and applying synthetic fertilizer to the foliage; not only are these methods minimally effective and/or costly, but there is little room for improvement (Spreen, Baldwin, & Futch, 2014). Despite the numerous benefits of synthetic fertilizer application, over-application of these products to the soil has led to negative environmental impacts (Munk & Hartz, 2017). For example, in California there has been increasing concern regarding the leaching of nitrates into groundwater and surface waters, causing environmental and public health concerns (Munk & Hartz, 2017). This has led to efforts by the government to establish regulations on nutrient management in agricultural operations, which aim to regulate fertilizer applications in order to mitigate these negative impacts (USDA, 2002). In California there have also been government financial incentives, such as the California Healthy Soils Initiative, which encourages farmers to adopt long term soil-building practices to promote soil health with organic inputs such as compost in order to offset synthetic fertilizer applications (USDA & CDFA, 2016). Given that the legislative environment is discouraging over-application of synthetic fertilizers, it is undeniable that there is a pressing need for farmers to adapt to these circumstances by finding alternative fertilization methods that conform to

tightening restrictions while simultaneously increasing opportunities to take advantage of financial incentives.

Based on the aforementioned challenges facing the citrus industry, there is definitely a strong need for effective solutions to ensuring citrus productivity beyond the current methods, as well as preventing pest problems without increasing pesticide applications.

Vermicompost Basics

Research in recent years regarding the benefits of vermicompost has proven to be an increasingly promising area of study towards finding a solution to these problems. There are numerous review papers that have compiled and summarized data from a wide range of studies (Jack & Thies, 2006; Joshi et al., 2015; Pathma & Sakthivel, 2012), and there are also several research publications that are particularly relevant to this topic that are worth mentioning.

The beneficial effects of vermicompost on nutrient uptake is well documented. Worm castings show potential in improving plant productivity by decreasing dependence on synthetic fertilizers because they are rich in stable organic matter and humic substances, slow-release plant-available nutrients, and a wide spectrum of various beneficial soil microorganisms including bacteria and fungi (Jack & Thies, 2006; Joshi et al., 2015; Pathma & Sakthivel, 2012).Vermicompost is also correlated with improved formation of soil aggregates, which improves soil structure by decreasing soil compaction and increasing water infiltration (Jack & Thies, 2006; Joshi et al., 2015; Pathma & Sakthivel, 2012), which is even promising with respect to mitigating some of the common drought problems that threaten California citrus growers. All of these aspects, as

reflected in a notable amount of research, have been associated with improved plant and soil health, increased plant growth, increased yields and productivity (Jack & Thies, 2006; Joshi et al., 2015; Pathma & Sakthivel, 2012).Worm castings can even help enhance the efficiency of chemical fertilizers when applied in combination (Joshi et al., 2015), thereby allowing the possibility of decreased synthetic fertilizer application rates. Vermicompost is also very promising in encouraging plant resilience against pests and diseases without the use of chemical pesticides; though the specific mechanisms are still not well understood, it has been associated with higher levels of disease resistance, as well as improved resistance to pest attacks after being applied either directly into soil or potting media, or from soil drench applications of aqueous extracts derived from vermicompost (Arancon et al., 2005; Edwards et al., 2010).

Although worm castings are a promising area of study, there is a wide range a variability when it comes to vermicompost, such as feedstock, worm species, and other details in the process of creating vermicompost (Aira, Olcina, Pérez-Losada, & Domínguez, 2016). Because of this variability, it would be beneficial to find a commercially available vermicompost product that has a standardized method of production that is readily available to be distributed on a commercial scale.

Despite the abundance of research across various plant varieties, there is limited research in the area of vermicompost and its effects on citrus trees (Joshi et al., 2015; Pathma & Sakthivel; 2012). Studying applications of vermicompost (such as direct application of worm castings to soil, or soil drenches and foliar sprays of aqueous solutions) and its effects on citrus tree health and protection against pest damage will provide useful information in developing practical applications.

Vermicompost Vs. Conventional Compost

Vermicompost is produced by the breakdown of organic waste by specific species of worms and microorganisms (Edwards, 2004, p.402-403). Earthworms play a vital role in conditioning and breaking down the organic matter into smaller pieces, thereby increasing the surface area for microorganisms to process it more quickly and more efficiently (Edwards, 2004, p.402-403). Through the process of moving through the earthworm gut, the biological, chemical, and physical aspects of organic matter are transformed (Edwards, 2004, p.402-403). The carbon to nitrogen ratio is decreased and beneficial microbial populations from the digestive tract of the earthworms is excreted in the earthworm casts (Edwards, 2004, p.402-403). The end result, vermicompost, is a porous, nutrient dense, stable product with a high water-holding capacity that has a wide range of benefits for plant growth, beneficial microbial populations, and soil health (Edwards, 2004, p.402-403).

Conventional composting relies on an optimum environment for microorganisms to break down organic matter, quickly and effectively converting it into a stable and pathogen-free organic soil amendment (Jack & Thies, 2006). However, the high temperatures reached in this process narrow down the bacterial populations to those which can survive the extreme heat, which have been observed to be mainly consisting of *Bacillus* and *Actinobacteria* (Dees & Ghiorse, 2001).

Vermicomposting does not reach high temperatures as it does in the standard thermophilic phases of regular composting processes; it remains in a mesophilic phase that maintains a higher diversity of microorganisms that are not destroyed by the high temperatures (Jack & Thies, 2006). It has also been shown to increase the nutrient quality

of, effectively stabilize, and eliminate pathogens in various organic matter substrates, despite the lack of temperature increase (Rajpal, Bhargava, Chopra, & Kumar, 2014).

Differences in Microbial Populations in Compost

The differences between the microbial populations of thermophilic compost and vermicompost are further emphasized in a study by Fracchia, Dohrmann, Martinotti, and Tebbe (2006), in which microbial populations of vermicompost were compared to thermophilic compost associated microorganisms. It was found that the microbial populations were very different, with genetic analysis retrieved from regular compost primarily belonging to the phyla *Actinobacteria* and *Firmicutes*. Vermicompost, on the other hand, was mainly populated with bacteria related to uncultured *Chloroflexi*, *Acidobacteria*, *Bacteroidetes* and *Gemmatimonadetes*. The authors emphasize the importance of variation in feedstock as a major factor in determining the bacterial populations in the finished compost product.

A similar study analyzed the difference among the bacterial composition of vermicompost produced from several feedstocks; horse, cattle, and pig manure (Aira, Olcina, Pérez-Losada, & Domínguez, 2016). In congruence with the previous studies, bacterial populations belonging to *Chloroflexi* and *Bacteroidetes* were found in vermicompost, in addition to the microbial populations that are characteristic of standard compost, such as *Firmicutes,* and *Actinobacteria.* Furthermore, the vermicompost proved to have a much richer bacterial population diversity with additional bacteria from the phyla *Proteobacteria*, and with lesser population density from the phyla *Verrucomicrobia, Hydrogenedentes, Latescibacteria, Planctomycetes* and *Candidatus Saccharibacteria*. The study emphasized the profound impact that feedstock has on the

outcome of the finished vermicompost, which was widely varied between the three substrates.

Stabilization of organic materials is the primary goal of composting, and many scientific research experiments have concluded that vermicomposting has shown improved rates of stabilization of nutrients above standard composting methods, as outlined in studies by Frederickson, Butt, Morris, and Daniel (1997) and Atiyeh, Domínguez, Subler, and Edwards (2000a). The experiment by Frederickson et al. (1997) was an analysis of the differences in green waste that was composted initially by the standard thermophilic procedure and subsequently removed at different stages of the process to be further processed by compost worms. The results indicated a strong positive correlation between the level of stabilized organic matter in the finished vermicompost and the earlier the compost was removed from the original thermophilic pile, indicating that the higher nutrient levels in unfinished compost led to more efficient breakdown by compost worms. Therefore, it was determined by the authors that thermophilic compost should be utilized for pathogen reduction only as long as necessary before being transferred to vermicomposting because of the enhanced ability of the worms to stabilize organic matter. Similar benefits of two-phase composting have been evidenced in other studies, which have concluded that various forms of organic matter were more stable after undergoing an initial thermophilic phase followed by a final vermicompost phase (Lazcano, Gómez-Brandón, & Domínguez, 2008; Tognetti, Laos, Mazzarino, & Hernandez, 2005).

Vermicompost Teas

There is a wide range of variability concerning the production and preparation of vermicompost and aqueous vermicompost solutions, and combinations of factors such as the type of organic material used as a feedstock, methods of processing, and worm species can produce a substantially varied results in the finished product (Scheuerell & Mahaffee, 2002). For example, as previously mentioned, Aira et al. (2016) compared bacterial communities in horse, cow, and pig manure and found that each substrate produced a drastically different bacterial population density in the finished product. There is also a wide range of variability with respect to aqueous extracts of vermicompost, such as aerated extracts in which a continuous flow of air bubbles is kept flowing through the mixture allowing oxygen to promote the growth of aerobic organisms, and non-aerated mixtures that are aqueous compost-saturated liquids that are left to ferment or used immediately (Scheuerell & Mahaffee, 2002). Direct application of vermicompost is yet another method and is simpler to prepare and utilize in the field or in greenhouse scenarios, such as in varying degrees of substitution in soilless potting media (Atiyeh, Edwards, Subler, & Metzger, 2000b).

Pathogen Suppression

Experiments involving vermicompost and various vermicompost aqueous solutions have been carried out with respect to control of various pathogens, as shown in a study in which aerated vermicompost tea suppressed inoculations of the pathogen tomato bacterial canker in combination with other biocontrol agents (Utkhede & Koch, 2004).

Other scientific studies have been performed to evaluate the gene diversity of vermicompost in comparison to fresh sludge as it relates to the suppression of the fungal *Fusarium moniliforme* pathogen via production of chitinase enzymes, such as the study performed by Yasir et al. (2009). Similar to the aforementioned studies, Yasir et al. (2009) found that bacterial populations from *Proteobacteria*, *Bacteroidetes*, *Verrucomicrobia*, *Actinobacteria* and *Firmicutes* were discovered. *Actinobacteria* was the most common in vermicompost in this particular case. Upon further analysis, it was determined that vermicompost also had the highest communities of chitinolytic bacteria. These specific bacterial populations that had a negative influence on the fungal pathogen were highest in vermicompost compared to fresh sludge, with chitinolytic isolates functioning as the most effective in limiting the growth of the pathogenic fungus (Yasir et al., 2009).

Effects on Pest Populations and Pest Damage

Arancon, Galvis, and Edwards (2005b) assessed the effects of substituting various levels of vermicompost at 0%, 20%, and 40% into a soilless potting medium and examined the levels of pest populations and damage by aphids, mealy bugs and cabbage white caterpillars on tomatoes, cabbage, and peppers. The study found that the substitution of vermicomposts into soilless potting media significantly suppressed populations of both aphids and mealy bugs on peppers, as well as mealy bugs on tomatoes. Observations also included significantly decreased losses of dry weights of peppers in response to both aphid and mealy bug infestations, in addition to significantly decreased losses in shoot dry weights of tomatoes after mealy bug infestations. There

were also significantly decreased losses in leaf areas of cabbage seedlings in response to the cabbage white caterpillar infestations.

In addition to reduction of pest attacks and populations with respect to direct application of vermicompost, there have also been notable findings with respect to application of aqueous solutions derived from worm castings, such as the study by Edwards, Arancon, Vasko-Bennett, Askar, Keeney, & Little (2010) that found that aqueous soil drenches of various concentrations of solutions made from worm castings also significantly reduced populations of green peach aphid, citrus mealybug, and two-spotted spider mite on tomatoes and cucumbers. Among the most relevant studies, as cited in the publication by Edwards et al. (2010), was one that found a beneficial effect of application of vermicompost on the suppression of *Heteropsylla cubana*, also known as the leucaena psyllid (Biradar et al., 1998), thereby indicating potential to suppress other insects from the Psyllidae family. This research is specifically relevant because it could have significant implications as far as protection of citrus nursery stock as well as commercial citrus orchards against ACP.

Effects on Plant Growth

Plant growth improvements are widely noted within the scientific literature regarding the beneficial effects of vermicompost (Joshi, Singh, & Vig, 2015; Pathma, & Sakthivel, 2012). Specific examples include a study in which vermicompost with high humic acid from trichoderma increased yields, mycorrhizal colonization and plant growth in pea plants (Maji, Misra, Singh, & Kalra, 2017), in addition to a study involving a notable improvement in greenhouse petunias in growth and flowering (Arancon, Edwards, Babenko, Cannon, Galvis, & Metzger, 2008). Improved yields are another

major effect of vermicompost applications as evidenced by several relatively recent studies, such as increases in pepper yields observed after vermicompost applications in a greenhouse setting (Arancon, Edwards, Atiyeh, & Metzger, 2004), and again in the field after vermicompost applications (Arancon, Edwards, Bierman, Metzger, & Lucht, 2005a). There were also notable improvements in marketable yields of corn, soybean, and lettuce from aerated compost tea solutions made from vermicompost and standard thermophilic compost (Kim, 2015). Bok choi yield also increased significantly under treatment with aerated vermicompost tea (Pant, Radovich, Hue, & Arancon, 2011). Furthermore, many studies have noticed an improved efficiency in fertilizer utilization, achieving the same or better yields by replacing up to 50% synthetic fertilizer with vermicompost without compromising soil quality (Jeyabal, & Kuppuswamy, 2001; Pathma, & Sakthivel, 2012). The aforementioned observations have very promising implications for applications to citrus cultivation.

Citrus Nursery Tree Production

Citrus nurseries are always seeking new methods of cultivating healthy, disease free trees that are resilient to adverse environmental conditions, which is a very important aspect to consider in order to ensure decreased susceptibility to pathogens and pest damage (Ferrarezi, 2019). After transplanting in the field, small trees are also weaker and more susceptible to heat, cold, and other environmental factors that make them vulnerable (Ferguson, Grafton-Cardwell, & University of California, 2014), thereby emphasizing the need to produce strong trees for replanting. To date, synthetic fertilizers are widely used to achieve this end (Ferrarezi, 2019).

Fertilizer Efficiency

Increased fertilizer efficiency is also another important aspect to consider when it comes to ensuring the productivity of citrus trees, especially since it has been demonstrated that heavy use of synthetic fertilizers can have detrimental effects on soil health (Mulvaney, Khan, & Ellsworth, 2009). With this in mind, environmentally friendly methods for plant growth and productivity are needed to reduce synthetic fertilizer inputs while maintaining current production levels.

Pesticide Usage

Pest problems have also been demonstrated to slow down the efficiency of plant growth by inhibiting basic processes, as evidenced by decreased photosynthetic rates in citrus trees affected by leafminer, for example (Schaffer, Peña, Colls, & Hunsberger, 1997). Other plant pathogens found in citrus nurseries that have detrimental effects on plant growth, such as *phytophthora,* have shown signs of fungicide resistance in the past (Timmer, Graham, & Zitko, 1998). This demonstrates that increased pesticide usage can have negative effects that are not yet fully understood when considering the entire microecology of plant defenses because there are many aspects of plant growth and health that are dependent on an extremely complex network of microorganisms (Pimentel, & Edwards, 1982). Pest management regimes that solely rely on heavy pesticide applications are becoming decreasingly viable (Dreistadt & University of California Integrated Pest Management Program, 2012, p. 33-35), both environmentally and economically.

Major Scientists in the Field of Vermicompost

Clive Edwards, author of Earthworm Ecology (2004), is known as one of the pioneers in studying earthworm interactions in soil and plant ecology. He effectively studied and explained many earthworm processes upon which so many vermicomposting principles are currently based worldwide (Edwards, & Fletcher, 1988). Edwards was primarily involved with Ohio State University, and several other authors have collaborated with him from the same institution (Arancon, 2004, 2005a, 2005b, 2006, 2008; Atiyeh 2000a, 2000b, 2002). Currently, many other institutions worldwide have begun to study vermicompost applications to the extent that it would be a difficult task to enumerate all of them.

Current Research Status on Vermicompost

Despite the abundance of research on vermicompost interactions with a wide range of different crops, there is limited research with regard to its effects on citrus trees (Joshi et al., 2015; Pathma & Sakthivel; 2012). There are some studies that have been carried out with respect to citrus and vermicompost, but they have mainly dealt with direct applications of vermicompost in soil media in combination with other amendments or plant growth promoting rhizobacteria, as well as mainly dealing with seeding of rootstocks (Yadav, Jain, & Jhakar, 2012; Suharsi, & Sari, 2014). As of yet, it seems that there is very limited research on the effects of aerated vermicompost teas on previously grafted scion-rootstock combinations. Due to this fact, and especially considering the economic importance of citrus, it would be most beneficial to help fill this research gap and provide valuable insight into an environmentally sustainable yet effective method for

the suppression of ACP and other pests, as well as sustainable methods for increased productivity and resilience in citrus orchards and nursery citrus stock.

Vermicompost Skepticism

Despite the majority of widespread praise for vermicompost as a new solution to plant productivity challenges, there is some disagreement about whether or not this is always the case. Tognetti et al. (2005) concluded that the superiority of vermicompost compared to regular compost is highly variable and dependent on the technology, feedstock and processes utilized, due to their mixed results comparing two vermicompost products with a conventionally composted product. Their study analyzed the effects of municipal compost broken down by the standard thermophilic process, municipal compost from the same source that was then processed by compost worms, and finally, a pure vermicompost made from a different feedstock and without a preliminary thermophilic phase. It was determined that the highest performing product was the municipal compost that underwent a thermophilic breakdown before being processed by worms, while the completely vermicomposted material was least beneficial.

In another study, it was found that vermicompost and regular compost didn't perform as well as mineral fertilizers when yields were compared (Doan, Ngo, Rumpel, Van, Nguyen, & Jouquet, 2013). By testing standard compost, vermicompost, and synthetic fertilizers separately in a greenhouse setting with a maize-tomato-maize cycle, vermicompost and compost did not yield as much as the conventional fertilizers when nutrient content was controlled. The authors stress the highly variable nature of composted products and the complexity of earthworm interactions as the probable reason for the negligible positive effects of vermicompost in this particular study.

Though there is a substantial amount of evidence suggesting that worm castings and vermicompost have numerous beneficial effects on plant growth, health, and productivity, there is still a great deal of debate with respect to what the specific mechanisms are that cause these benefits (Jack & Thies, 2006; Joshi et al., 2015; Pathma & Sakthivel, 2012).

Humic Acid: An Important Component of Compost

Humic substances are a noteworthy aspect of properly stabilized organic matter (Atiyeh, Lee, Edwards, Arancon, & Metzger, 2002), and due to this important aspect of effective stabilization of organic matter through composting, considerable efforts have been made in attempting to increase humic acid levels in vermicompost with notable success (Maji, Singh, Wasnik, Chanotiya, & Kalra, 2015). When considering the observed benefits on plant health that are associated with humic substances, it is clear that it is a topic worth delving into.

A scientific investigation by Canellas, Olivares, Okorokova-Facanha, and Facanha (2002) determined that, although not fully understood, plant growth is thought to be attributed to the various mechanisms in humic acid in improving the availability of plant hormones such as exchangeable auxin groups. In this study, humic isolates were extracted from vermicompost and showed an increase in lateral root emergence and overall root growth in maize. In aqueous vermicompost exracts, Zhang et al. (2014) extracted humic substances high in cytokinins, which are other phytohormones responsible for improved plant growth and productivity. The association of these phytohormones with humic substances is very promising and has provoked other scientific studies in this area. For example, Maji et al. (2017) utilized a novel strain of

trichoderma fungus to roughly double the humic acid content of vermicompost and subsequently tested the results on several different aspects of plant growth and productivity of pea plants. They observed increases in yield, rhizobium bacterial colonization, mycorrhizal colonization, as well as overall plant growth, thus promoting the legitimacy of the theories regarding humic acid as a crucial component of plant health.

Arancon, Edwards, Lee, & Byrne (2006) carried out a study to compare commercially produced humic acid and commercially produced IAA with those derived from vermicompost at different rates to see if there was any difference between them. They performed the experiment on several different plant species, being strawberries, peppers, and marigolds. It was determined that the vermicompost derived humic acids increased pepper flowering and fruiting more than the commercially available products, indicating that there may be aspects of vermicompost derived humic acids that outperform the commercially produced phytohormones and humic acid products. Humic acid extracted from vermicompost was also shown to improve growth in tomato and cucumber seedlings in soilless potting media (Atiyeh et al., 2002). However beneficial these substances may be, an important question to ask is whether or not humic acids with beneficial phytohormone effects can be extracted from vermicompost teas and whether or not they are water soluble. Atiyeh et al. (2002) used a more complex method to extract humic substances, possibly indicating that a simpler aerated vermicompost tea may not contain some of these beneficial humic substances and associated phytohormones.

Beneficial Microorganisms

In addition to phytohormones, it is well established that certain microorganisms can greatly improve plant health, as demonstrated in a study by Vessey (2003) that categorizes the roles of plant growth promoting rhizobacteria in the basic categories of modes of action including dinitrogen fixation, improving nutrient availability in the rhizosphere, having a positive impact on root development and morphology, as well as creating more opportunities for other beneficial plant–microorganism symbioses. These rhizobacteria have also been associated with decreased susceptibility to pathogens by promoting induced systemic resistance for increased pathogen resilience (Pieterse et al., 2003).

Potential Pathogen Risks of Vermicompost

Pathogen transmission is also another major concern to consider when utilizing any composted product, specifically because they contain naturally processed organic matter (Noble, & Roberts, 2004). It is important to consider the methods used to transform organic matter and ensure adequate levels of pathogen destruction (Noble, & Roberts, 2004). Common thermophilic composting methods solve this problem by reaching a specific temperature in order to eliminate unintended pathogens (Noble, & Roberts, 2004); however, vermicomposting relies on a different mechanism. There is strong evidence indicating natural pathogen destruction in vermicomposting processes through the coelomic fluid present in earthworm guts, which has been correlated with significant reductions in certain phytopathogenic fungi (Plavšin, Velki, Ečimović, Vrandečić, & Ćosić, 2017), and has also been shown to substantially reduce or eliminate pathogenic bacterial populations in the most pathogen-dense substrates such as human

waste biosolids (Yadav, Tare & Ahammed, 2010). Furthermore, it was demonstrated to significantly reduce pathogens inoculated into feedstock (Eastman et al., 2001) and to reduce fecal coliforms (Rajpal et al., 2014; Edwards, 2004, p.414).

However, during the process of making vermicompost tea extracts, some caution is advised when working with nutrient sources, as outlined in a study in which both aerated and non-aerated compost teas did not increase incidence of salmonella and *e.coli* when no nutrient source was added, but both methods did increase *e.coli* and *salmonella* when a nutrient source was utilized (Ingram & Millner, 2007).

Aerated Vs. Non-Aerated Vermicompost Teas

Aerated compost teas are thought to promote beneficial aerobic bacteria, and many believe that they are superior to non-aerated brews (Scheuerell & Mahaffee, 2002). In a scientific study analyzing the beneficial microbial populations of aerated regular compost brew, it was found that maximum soil microbe density in solution was within 2 days of brewing and that aeration greatly improved beneficial microbial populations (Islam, Yaseen, Traversa, Ben Kheder, Brunetti, & Cocozza, 2016).

Non-aerated aqueous extracts derived from various substrates have been proven to reduce damping-off pathogens in vitro (Koné, Dionne, Tweddell, Antoun, & Avis, 2010). Increases in okra productivity from non-aerated vermicompost liquid extract, termed "vermiwash" in this particular experiment, was also reported (Ansari & Sukhraj, 2010). Non-aerated regular compost tea was also shown to have positive effects on pathogen resistance against *Botrytis cinerea* on beans and lettuce (McQuilken, Whipps, & Lynch, 1994).

Nutrient Sources in Compost Teas

Adding nutrient sources in compost aqueous extracts is also an important topic of disagreement. Scheuerell & Mahaffee (2002) emphasize that there is a strong acceptance among compost tea users that nutrient sources exponentially enhance the beneficial microbial communities. A separate study by Scheuerell & Mahaffee (2004) found that molasses-based nutrients provided an inconsistent pathogen suppression response, which indicates that the nutrient source can also become a potential substrate for pathogenic microorganisms. However, in the same study, they did find that aerated compost tea with kelp and humic acid nutrient sources provided reliable and effective pathogen suppression. This raises further questions about the quality and type of nutrient source in compost teas.

Also, taking into account the previously mentioned study in which both aerated and non-aerated methods of compost tea production did increase *e. coli* and *salmonella* when a nutrient source was utilized, it seems that these pathogens can also piggy-back onto the nutrients meant for beneficial microbes in order to multiply (Ingram, & Millner, 2007). With this in mind, adding a nutrient source should be done cautiously or avoided when working with questionable or possibly contaminated compost samples, especially seeing as there is evidence that regular aerated compost tea prepared without a nutrient source still suppressed pathogens and is therefore possibly unnecessary (Palmer, Evans, & Metcalf, 2010).

Methodology for conducting plant analyses in this area

Methodology in evaluating effects of vermicompost applications would include measuring shoot growth and root growth, which is a reliable to way determine plant

growth within a given period of time, as performed in studies by (Brar & Spann, 2014; Maji et al., 2017; Arancon et al., 2008). In the aforementioned studies, plants were harvested, and shoots and roots were separated, dried and weighed for biomass quantification. When studying small citrus trees, this seems to be an effective way to accurately measure root and shoot growth.

Experimental design for studying citrus treatments was well exemplified in a study by (Sharma, Dubey, Awasthi, & Kaur, 2016), in which a total of 45 trees were examined from 9 different rootstocks in a randomized block design. Each rootstock had 5 replications under different groups, which effectively demonstrated a proper random distribution of potential confounding effects.

For pest densities, tap sampling and visual observations in a repeated measures experimental design has been performed for ACP and leafminer studies in related research (Stansly & Kostyk, 2018).

EXPERIMENTAL DESIGN, METHODOLOGY, AND PARAMETERS

Objectives

The objectives of this study are to determine the effects of aqueous vermicompost solutions on pest densities and vegetative plant growth in citrus nursery trees. Specific objectives with regard to biomass will consider measurements in leaf biomass, shoot biomass, root biomass, and trunk diameter. Specific objectives with regard to pest densities will analyze populations of ACP, leafminer, and thrips over the course of several months.

Experimental Design

The experimental design consisted of four groups of potted navel orange trees, approximately one year old, with 20 replications in each group. The first group served as a control group and did not receive an aqueous vermicompost treatment. The second group received a soil drench of eight fluid ounces of aqueous vermicompost solution, the third group received a foliar spray of aqueous vermicompost solution with an organic surfactant, and the fourth treatment group received the same soil drench treatment and the same foliar treatment combined. All trees were watered and fertilized equally.

There were three different configurations throughout the course of the experiment. The first configuration was separating the trees by treatment group within a greenhouse setting while they were in one-gallon plastic pots from July 5, 2018 until August 8,, 2018. The second configuration was the same and lasted from August 8, 2018 until October 29,2018; the only differences were that they were relocated outside of the greenhouse. The final configuration of the experiment was placed in the same location and used the same water source, but the trees were re-potted on October 29, 2018 in

larger pots that were spaced more widely apart. Starting in the month of November, the trees were randomized monthly in a completely randomized design. All 80 trees were placed in eight rows of 10 trees each. At this point, trees were labeled with individual numerical tags for each tree. Control treatment group received numbers 1-20, soil drench treatment group received numbers 21-40, foliar spray treatment group received numbers 41-60, and soil drench plus foliar spray treatment group received numbers 61-80. Each month, Microsoft Excel was used to generate a plan to randomly redistribute the trees. The completely randomized design was used to reduce effects caused by uneven light exposure, increased insect exposure on the edges, overspray from nearby irrigation, and other factors. Moving the trees each month also had the added effect of ensuring that the tree roots did not penetrate the soil beneath the mesh pots, altering the reliability of the data collected at the end of the study. Pest data was taken monthly.

Methods and Materials

The experiment consisted of a sample size of 80 potted citrus trees of the Thompson Improved navel orange cultivar on Carrizo rootstock. The citrus trees were initially grown in a cylindrical coir-like media wrapped in a weed cloth-like disposable fabric. On July 05, 2018, the disposable fabric material was removed, and the trees were then re-potted in 5”x5”x12” (approximately one-gallon) black plastic pots for improved stability and Kellogg® brand potting soil for palm, cactus, and citrus was used to fill in the remaining space in the pots. The pots were labeled with each treatment group, C for control, S for soil drench, F for foliar spray, and F+S for combined soil drench and foliar spray. These trees were placed in a greenhouse on the campus of California State Polytechnic University, Pomona.

On August 08, 2018, the potted trees were re-located outside within 100 feet of the same greenhouse to examine native pest populations. A small support structure was constructed out of PVC and plastic cord in a grid formation to keep the pots upright and to support the irrigation structure that was established. Additionally, colored cable ties were loosely attached around the trunk of each tree in order to color-code each treatment group for ease of identification. The control group was labeled with blue cable ties, the soil drench group was labeled with yellow cable ties, the foliar spray group was labeled with orange cable ties, and the combined foliar spray and soil drench group was labeled with pink cable ties. The drip irrigation system was placed on an Orbit® 62061Z one-outlet programmable hose faucet timer, a Renator® M11-0660R water pressure regulator valve, a two-stage HydroLogic® HL36010 Tall Boy carbon water filter with upgraded KDF85 catalytic carbon filter to ensure adequate reduction/removal of chlorine from municipal water treatment. Each tree had one 0.5 gallon per hour emitter, for a total of 80 emitters.

On October 29, 2018, the trees were eventually re-potted in flexible pots made from an elastic mesh provided by Filtrexx® to encourage oxygen exposure and increased root growth as the plants developed. Filtrexx® was cut into two-foot segments and tied off at one end with a cable tie to create pots of a larger size. The flexible nature of the material expanded horizontally more than anticipated, leaving the top of the root ball slightly exposed. On November 20 and 21, 2018, each tree was re-potted with another layer of Filtrexx® mesh outside of the first layer, this time in four-foot segments due to an increased need for covering the top of the root ball so that more potting soil could be added. Weed cloth was placed underneath the trees to ensure that the roots did not

penetrate the soil underneath the pots. Shortly after the trees were re-potted, more extensive irrigation tubing was established on November 2, 2018. This irrigation setup still utilized one 0.5 gallon per hour drip emitter per tree. On November 21, 2018, it was modified to include one additional 0.5 gallon per hour drip emitter per tree, for a total of two 0.5 gallon per hour drip emitters per tree. This step was taken for improved wetting of the root zone due to larger pots. Irrigation time was adjusted accordingly to accommodate the additional emitter. Irrigation water was periodically tested for total chlorine content with a colorimeter for sufficiently precise measurements. As time progressed, it was necessary to add additional in-line carbon filters, which were ShurFlo® RV-210GH-A Waterguard filters. Five additional inline filters were added throughout the course of the study at approximately every two months. The apparatus used to test total chlorine levels was a Hanna Instruments® HI711 Checker® HC. This device was designed to test for total chlorine levels, which includes volatile free chlorine as well as chlorine that is bound to other molecules in the water source. It reports values in parts per million (ppm) of total chlorine.

Treatments throughout the experiment consisted of four groups; 20 plants received a systemic soil drench of WormGold® vermicompost extract, 20 plants received a foliar spray of WormGold® vermicompost extract combined with Therm-X70® surfactant, 20 plants received both a systemic soil drench with WormGold® vermicompost extract and a foliar application of WormGold® vermicompost extract combined with Therm-X70® surfactant, and 20 plants served as a control group. Each treatment was applied at two-week intervals for the first three treatments per the WormGold® application instructions, and subsequently once a month throughout the rest

of the experiment, which lasted 12 months, from July 5, 2018, to August 13, 2019. 13 total WormGold® treatments were applied, on July 31, 2018, August 15, 2018, August 29, 2018, October 2, 2018, November 1, 2018, December 4, 2018, January 4, 2019, February 1, 2019, March 7, 2019, April 10, 2019, May 2, 2019, June 4, 2019, and July 2, 2019.

The preparation of WormGold® vermicompost aerated aqueous solution that was used throughout the following experiments was prepared per the manufacturer's instructions. The WormGold® mix kit is a semi-permeable disposable cloth bag filled with worm castings which must be soaked for 24 hours with approximately eight ounces of non-chlorinated water to prime the dry material for the aerated brewing process. The amount of water was enough to moisten the bag of worm castings without dripping from the bottom of the bag when it was lifted off of the bottom surface of the five-gallon bucket in which it was resting. The brewing equipment consists of a 25-gallon container, in this case a brand-new Rubbermaid® trash can, fitted with aeration components constructed and provided by WormGold® to circulate air bubbles throughout the solution. This apparatus was filled with 25 gallons of non-chlorinated water. In order to ensure high quality water for the brewing process, five-gallon plastic water jugs were used to acquire drinking-quality water, which was tested for chlorine before the first brew at 0.00 parts per million. Water for every WormGold® preparation was acquired at the same establishment. The nutritional source for compost microorganisms was provided by adding a manufacturer recommended quantity of molasses dissolved in one gallon of lukewarm water into the brewing equipment. The brewing equipment was then turned on in order to circulate the mixture evenly. After the nutritional source was added, the pre-

soaked bag of worm castings was submerged and suspended in a perforated basket to allow water and air bubbles to flow through the bag. The mixture was left to brew for 24 hours at approximately 80 degrees Fahrenheit. Temperature was measured by means of a submersible probe thermometer suspended in the solution and recorded at the beginning and at the end of the brewing process. This mixture was used immediately after brewing for optimum effectiveness (Scheuerell & Mahaffee, 2002).

For foliar sprays, WormGold® vermicompost extract was combined with Therm-X70®, an organic wetting agent made from yucca plant extracts that served as a surfactant to decrease surface tension and improve coverage on the leaf surface (Kjellin, Johansson, & Johansson, 2010). 1,538 mL of the WormGold® vermicompost solution was added into the handheld pump sprayer along with 6,033 mL of purified, non-chlorinated water. A small measuring spoon was used to add five mL of surfactant to the mixture in the pump sprayer reservoir and agitated by hand to mix. Solution was applied with a hand-pump sprayer individually to each tree that required a foliar application.

A standard granular 6-4-6 citrus fertilizer, Vigoro® Citrus and Avocado Food, was used to ensure adequate nutrient availability to the trees throughout the growing process. One half teaspoon of fertilizer were applied to each pot on July 09, 2018, August 06, 2018, September 10, 2018, and October 26, 2018. The trees were subsequently re-potted on October 29, 2018 with fertilizer amended potting soil. The potting soil was a custom blend containing 15 pounds of 15-6-12 slow release fertilizer, five pounds of 0-45-0 fertilizer, two pounds of 1-0-1 fertilizer with iron, and 16-16-16 fast-release fertilizer, all mixed into 27 cubic feet of potting soil. Not all of the potting soil was used to fill the pots. Because of the combination of fertilizers added to the potting medium and

the slow fertilizer uptake during the cool season, the next fertilizer application of one half teaspoon of Vigoro® Citrus and Avocado Food was not applied until March 06, 2019. An additional two and a half teaspoons were applied on April 25, 2019 due to minor foliar deficiency symptoms.

All trees were marked with specific tags indicating which of the four vermicompost treatments it would receive. The amount of solution applied to the root zone for soil drench applications was determined by using a standard measuring cup. The quantity of WormGold® applied as a soil drench to each tree was 237 mL.

All the trees used in this experiment were obtained from Young's Nursery in Thermal, California. The trees were grafted approximately one year before they were acquired for this experiment.

Data Collection and Analysis for Biomass

The parameters measured at the end of the study included dry root biomass, which was determined by removing potting medium, drying, and weighing root mass, as well as above-ground biomass consisting of shoots, leaves, and stems, which were also dried and measured by weighing.

Upon completion of the 12-month growth period, on August 13, 2019, 10 trees from each treatment group were selected for biomass analysis. The trees selected for the control group were numbered 11-20 because they did not receive an accidental soil drench treatment as trees 1-10 did (see "Limitations of This Study" section). The other three treatment groups were randomly selected. When trees were determined, harvesting clippers were used to separate the root mass from the trunk and shoot mass. After separating the roots from the shoots, the trunk and shoots were cut into several pieces in

order to fit into paper grocery bags that were labeled with the tree number, date, treatment group, “M. Lasiter,” and “shoots” to indicate that the bags contained above-ground biomass. The paper bags with above-ground biomass were left under a tarp outside overnight until they could be placed in a drying oven the following day. The root biomass was also left in the potting medium in the same place until the following day.

Root biomass was separated in several stages. The first stage was conducted on August 14, 2019 and involved the removal of the mesh pots from the potting media and the removal of all the loose potting soil around the root ball. The mesh pots and loose potting media were discarded. The root balls were labeled with their original tags and set aside on a tarp, with a protective tarp over the top. While the root balls stayed protected under the tarp, the leaf and shoot biomass prepared the previous day was taken to a drying oven in the Biology Department at Cal Poly Pomona. Each of the 40 specifically labeled bags was placed in the drying oven at 70 degrees Celsius for 14 days, or approximately 336 hours.

The second stage of root separation, started on August 16, 2019, involved using a piece of hardware cloth over a shallow plastic tray which acted as a screen to catch the roots and allow the potting media to fall through. This was the primary method to separate the root mass from the potting media, and each root ball was pulled apart and separated by hand. The separated roots were placed in paper bags labeled with the date, tree number, “M. Lasiter,” and “roots.” When finished, the bags were set outside on a tarp over the weekend in order to dry until they could be placed in the drying oven the following week. On August 19, 2019, at approximately 1:30PM, the roots were placed in a refrigerator in the Agriculture building at Cal Poly until root samples could be taken for

pathogen sampling on agar media. On August 21, 2019, the root samples were taken out of the refrigerator, and 1-inch samples were taken from several roots. The root biomass samples were then re-bagged and placed back in the refrigerator. It was later determined that the root pathogen aspect of the study was unable to be completed within the given time frame, and therefore the root samples will not be discussed further. The roots remained in the refrigerator until August 28, 2019, when they were taken to the drying oven in the Biology Department at Cal Poly Pomona. The above ground biomass that was in the drying oven was removed and replaced with the root biomass paper bags. The drying oven was still set at a temperature of 70 degrees Celsius, and the root biomass remained in the oven for 12 days or approximately 288 hours.

The papers bags that were removed from the drying oven containing the above ground biomass were transported to the soil lab at Cal Poly Pomona. The first stage of analysis involved separating the dried leaves from the shoots over an aluminum pan. Each of the 40 specimens were individually separated over the aluminum pan to separate leaves from shoots, and then placed back in the bag. After this was done for every replication, the bags were moved to an area where they could be weighed. A digital scale was utilized to take appropriate biomass measurements. Two aluminum pans were used for separating the biomass. First, one aluminum pan was placed on the scale and the "tare" button was pressed to zero out the scale. All of the shoots were then removed from the bag and placed on the aluminum pan on the scale. The reading was allowed to stabilize and was recorded. Then, the dried shoots were moved to a separate aluminum pan to the side. The same aluminum pan was used on the scale, and it was verified that it

reached a reading of 0.00g before adding the remaining leaf biomass. Once leaf biomass was recorded, it was placed back in the appropriately marked paper bag.

A digital caliper was then used to take trunk diameter measurements by locating the trunk in the aluminum pan containing the dried shoots. The digital caliper was set to measure in millimeters and was placed approximately one inch above the cut, which was cut directly at the soil line. The trunk was measured from the widest point. After the trunk diameter measurements were recorded, the shoot biomass was replaced into the original appropriately marked paper bag for storage. This process was repeated for all 40 samples.

For the measurement of root biomass, the roots were removed from the drying oven on September 09, 2019, at approximately 10:00AM. They were transported to the lab in the Agriculture building at Cal Poly Pomona to be weighed. The aluminum pan used in the previous shoot and leaf mass measurements was used again in this procedure. It was placed on the scale, the "tare" button was pressed until the scale read 0.00g. The contents of the root mass samples were emptied into the tray and recorded, and then the root mass was placed back into the bag and set aside. This process was repeated for all 40 samples.

All biomass data were analyzed using the statistics program SAS® software (SAS Institute Inc., Cary, NC, USA). They were entered into the system in a one-way ANOVA with alpha level set at 0.05 significance.

Data Collection and Analysis for Pests

Pest densities were evaluated for three major common pests, including thrips, leafminer, and ACP. Pest densities were recorded once monthly on ten randomly selected trees from each treatment group. The randomized selection was different each month, but

pest densities for thrips, ACP, and leafminer were all taken from the same randomly selected trees each month.

For thrips, three separate flush points on the tree were selected for sampling. On each flush point, a pen was used to tap each flush point exactly three times over a clipboard with a piece of white printer paper for ease of visibility. Any thrips that fell onto the paper were counted and recorded. If identification was needed, a small hand lens was used to identify the insects. This process was repeated once monthly from February until July. February data were not included in the final analysis due to pest identification complications. The first round of data for February included thrips counts that were later identified as Collembola spp.

For ACP populations, it was initially determined to count eggs, nymphs, and adults on five flush points per tree. Tap sampling and visual observations were used to gather ACP counts. The tap sampling procedure was exactly the same as that for thrips; three taps with a pen over a clipboard for each flush point. With ACP however, 5 flush points were sampled. Because of the age of the citrus trees, a limited amount of flush points was available on each tree. For this reason, the first three flush points used in the thrips data collection were also used as the first three flush point samples for ACP data collection. If any adult ACP were observed in the first three flush points, the number of psyllids was recorded on the ACP data sheet. After the first three flush points were sampled, an additional two flush points were tap sampled with the same 3-tap per flush point procedure. After collecting all of the data for the season, not a single nymph or egg was found. For that reason, in the final data analysis, only adult ACP counts were

included. The ACP populations during the season in which this study was carried out were notably low, which undoubtedly affected the data collection in this study.

Methods for leafminer sampling were to randomly select 10 leaves per tree to observe. Leaves that showed incidence of leafminer were recorded. Dividing the number of leaves mined by the total number of observed leaves provided a clear method of determining the percentage of leaf damage on the tree.

The pest data were conducted as a repeated measure of a completely randomized design and were analyzed in the statistics program SAS® software (SAS Institute Inc., Cary, NC, USA). statistics program in a two-way ANOVA, considering treatment and time as important factors with potential interactions between groups.

RESULTS AND DISCUSSION

Biomass Results

Leaf Biomass

Leaf biomass included only the biomass of dried leaf tissue separated from the shoots. It was determined that leaf biomass of all treatment groups was insignificantly different from the control group; the probability of treatment effect was 0.1172 with alpha set at 0.05 (Table 1, Fig. 1). However, the mean biomass followed a trend, with the foliar spray treatment group with the most biomass at 49.82 grams, soil drench treatment group at 41.37 grams, soil drench combined with foliar spray at 41.2 grams, and the control group with the least biomass at 39.02 grams.

Shoot Biomass

Shoot biomass included the biomass of the trunk, stems, and shoots of the citrus trees, excluding root mass and leaf mass. The probability of treatment effect was 0.1141 with alpha set at 0.05 (Table 2, Fig. 2). Because of this result it was determined that shoot biomass cannot be considered significantly different. However, when considering mean biomass trends alone, the foliar spray group had the highest mean shoot biomass at 79.73 grams, with soil drench at 74.89 grams, followed by soil drench combined with foliar spray group at 69.14 grams, and the control group with the least mean biomass at 67.37 grams.

Root Biomass

Root biomass was very close to reaching significance, with the probability of treatment effect equaling 0.0562 with an alpha level of 0.05 (Table 3, Fig. 3). It is not unreasonable to assume that this treatment could have surpassed the significance

threshold with a higher sample size. The treatment in the highest t group was the foliar spray group, which had a mean root mass of 125.89 grams. All other treatments were in the second highest t group: 91.37 grams for the soil drench group, 89.67 grams for the soil drench and foliar spray combined group, and 82.35 grams for the control group.

Trunk Diameter

The probability of the treatment effect for trunk diameter was 0.2436 with alpha set at 0.05 (Table 4, Fig. 4). Although this was not considered to be a significantly different measurement, it did continue to follow the biological trend, with highest trunk diameter found in the foliar spray treatment group at 18.31 millimeters, soil drench group at 18.17 millimeters, the soil drench combined with foliar spray group at 17.53 millimeters, and finally the control group with the lowest trunk diameter at 16.86 millimeters.

Pest Density Results

Asian Citrus Psyllid

A two-way ANOVA was performed on ACP population data gathered over six months, from February to July, which was configured as a repeated-measures in a completely randomized design in which treatment factor and month were the repeated-measures factors. The results of the two-way ANOVA determined that there was no overall significance in ACP populations, with the probability of overall treatment effect equaling 0.4672 with an alpha level of 0.05 (Table 5, Fig. 5). The mean overall ACP populations were 0.15, 0.1, 0.08, and 0.07 ACP per tree for soil drench combined with foliar spray group, control group, foliar spray group, and soil drench group, respectively. The probabilities of the month effect, as well as the month x treatment effect were 0.0168

and 0.5376, respectively, also with an alpha level of 0.05. This indicates that there was an overall significant effect of the month on pest populations, which is apparent in the mean populations by month, being 0.225, 0.175, 0.075, 0.050, 0.025, and 0.050 ACP per tree for the months of February, March, April, May, June, and July, respectively.

Thrips

For thrips populations, 5 months of data were used in the final analysis, being March through July, in a two-way ANOVA. The probability of the overall treatment effect was 0.4784 with an alpha level of 0.05 (Table 6, Fig. 6). Mean overall populations were determined to be 0.72, 0.48, 0.44, and 0.42 thrips per tree for control group, soil drench group, foliar spray group, and soil drench combined with foliar spray group, respectively. The probabilities of the month effect, as well as the month x treatment effect were <0.0001 and 0.6940, respectively, also with an alpha level of 0.05. This indicates that there was an overall highly significant effect of the month on pest populations, which is apparent in the mean populations by month, being 0.025, 0.1, 0.6, 1.075, and 0.775 thrips per tree for the months of March, April, May, June, and July, respectively.

Leafminer

Leafminer populations were analyzed over the months of May, June, and July, and it was determined that there was an overall significant difference between the treatment groups using a two-way ANOVA, with the probability of overall treatment effect equal to 0.0240 at an alpha level of 0.05 (Table 7, Fig. 7). There was no statistical significance in the pest populations between months alone, possibly because only three months were used as opposed to five and six months for the thrips and ACP, respectively; the probability of month effect was equal to 0.2837 and the probability of treatment x

month interaction was equal to 0.3281. Upon performing the multiple means comparison procedure (Fisher's LSD t-test), it was determined that the control group had statistically higher overall leafminer populations than the three treatment groups. Mean overall leafminer populations were 0.63, 0.27, 0.27, and 0.17 leaves mined per tree for control group, foliar spray group, soil drench combined with foliar spray group, and soil drench group, respectively. In particular, leafminer populations in May were statistically significant between treatment groups, with the probability of treatment effect equaling 0.0016, while June and July were determined to be statistically insignificant at probabilities of 0.2815 and 0.3802, respectively.

Discussion

Although the biomass data were not statistically significant, there was a notable and consistent trend in overall biomass results. Foliar spray treatment had the highest mean biomass and mean trunk diameter, soil drench had the second highest mean biomass and mean trunk diameter, soil drench combined with foliar spray had the third highest mean biomass and mean trunk diameter, and control group had the lowest mean biomass and mean trunk diameter. The means across treatment groups were ranked in the same order across shoot biomass, leaf biomass, root biomass, and trunk diameter. It is not unreasonable to assume that the root biomass could have surpassed the significance threshold with a larger sample size, which may provide valuable insight for future studies.

The question of whether the Therm-X70® surfactant had an impact on the overall treatment is also another important aspect to address. The foliar spray group and the soil drench combined with foliar spray group both received a diluted form of the

vermicompost solution mixed with the surfactant, and the soil drench combined with foliar spray group did not seem to show the same amount of growth that was observed in the treatment group that received a foliar spray treatment alone. This most likely indicates that the difference in the treatment was not due necessarily to the presence of the Therm-X70® surfactant, but rather the lower concentration of WormGold®. The unique effects of the foliar treatment alone may have occurred because it was the only treatment group to receive a very diluted amount of the treatment. Because the other two treatment groups received a soil drench of 237mL of pure vermicompost solution, it seems reasonable to conclude that a much more diluted application may be beneficial and that there are diminishing returns as far as higher concentrations are concerned. Needless to say, higher effectiveness at low concentrations is a positive aspect considering the fact that large agricultural and horticultural operations need to apply large quantities of treatments, striving to keep costs to a minimum.

Unfortunately, ACP populations were low during the season in which this experiment took place, leading to data that were not ideal. There were high counts of zeros in the raw data set, and only adults were observed on the trees, which can be more difficult to observe than nymphs. In order to further study the effects of vermicompost applications on ACP populations, it seems necessary to create a much more controlled environment that measures ACP-related parameters exclusively. The broad focus and time limitations of this study were insufficient to derive any significant conclusions.

Results of data analysis on thrips populations were also not significant. As previously stated with regard to ACP, further study of the effects of vermicompost applications on thrips populations would be beneficial in a much more controlled and

specific study. The broad nature and limited time frame of this study were not focused enough to determine any significant conclusions.

Leafminer populations, on the other hand, were significant. Although the significance was found in the month of May exclusively, it may suggest that there is an interaction between vermicompost applications and leafminer populations that is worth pursuing in greater depth. It seems, from the results of this data, that there may be an effect in which applications of aerated aqueous vermicompost solutions to citrus trees may delay the onset of leafminer colonization in a given season. If this happens to be the case, it could be a valuable strategy in integrated pest management approaches for keeping thresholds below a certain level.

CONCLUSION

Limitations of this study

There were several aspects of this research study that were limited, and there were also some unforeseen challenges. As previously mentioned, the addition of chlorine in municipal water sources ensures adequate destruction of potentially harmful microorganisms in drinking water. For this same reason, the presence of chlorine in municipal water sources can adversely affect the beneficial microorganisms that are such an important aspect of this study. Unfortunately, we did not have access to irrigation water from an untreated source, such as a well. In order to accommodate this municipal water source, carbon filters had to be used to remove the chlorine. The irrigation water was tested periodically to keep chlorine levels as low as possible, but it was impossible to keep the chlorine levels down to zero with our limited resources. The total chlorine levels present in the irrigation water varied between 0.01ppm and 0.16ppm depending on when new carbon filters were added, compared to a sample from a nearby faucet from the same water source, which tested at an average of 0.26ppm. It is still uncertain to what degree the chlorine levels negatively impact the survival of microorganisms in aerated aqueous vermicompost solutions, but the minor presence of chlorine in the irrigation water, even though it was substantially reduced, may have affected the results of this study.

Filtrexx® pots were great for air circulation, but drip emitters made it difficult to get adequate moisture around the edges of the pot. Because of this aspect, the potting media was perpetually dry around the periphery of the pots, potentially affecting root development by confining it closer to the center of the pot. However, drip emitters were

necessary to prolong the duration and effectiveness of chlorine removal by the carbon filters by reducing the volume of water passed through the filters. Ideally, irrigation by means of micro-sprinklers would be much better to ensure adequate moisture around the entire pot because, unlike solid plastic pots, water is able to permeate the mesh material into the potting media from the outside, thus keeping the potting media moist yet not water-logged and encouraging outward root development.

It was also difficult to keep the amount of the potting medium consistent between tree containers because of the flexible nature of the Filtrexx® material. In retrospect, it would have been useful to ensure consistency of the amount potting media placed in each pot when transferring the trees into the Filtrexx® containers by pre-measuring a specific weight or volume.

Re-potting the trees twice proved to be challenging because of tree stress. Trees were stressed after the second re-potting and showed signs of water stress and chlorosis afterwards for several weeks. When temperatures increased in the spring, and transpiration and fertilizer uptake increased, the trees began to improve. However, this could have affected the results of the experiment. Re-potting the trees also made it very difficult to separate the root mass from the potting media, since the nursery trees were propagated in a coco-coir-like media that was very difficult to separate the roots from. In future experiments, it would be beneficial to start the trees in one pot with one homogeneous potting medium that would be more conducive to separating from root mass.

Fertilization was another aspect of this study that could be improved in future experiments. A granular fertilizer was applied to the surface of the potting media

periodically to ensure adequate plant nutrition throughout the course of this study. The problem with this approach, as combined with drip emitters, is that the granular fertilizers have to come in contact with the moist potting media immediately around the drip emitter. This may cause variations in dissolution rate, mass flow, and plant uptake rates of these granular fertilizers. Many large-scale nursery approaches to citrus tree fertilization are a fertigation approach because supplying nutrients in parts per million through the irrigation water is much more precise and efficient (Guazzelli, Davies, Ferguson, & Castle, 1995). Though this method is much more precise and consistent, it was not feasible for this particular study due to specific limitations.

An error was also made during one of the soil drench applications in which 10 trees accidentally received a soil drench treatment in the control group. These trees were identified and marked accordingly, and in the final biomass analysis of a subsample of 10 trees, they were not included. However, they were included in random pest density repeated measures analysis. This error accounted for only one out of the 13 treatments, only affected half of the control units, and was only relevant to pest density data, but it is nonetheless still necessary to document this occurrence.

Future directions of research

Additional or parallel research that would complement and expand on this study would include several major components, including but not limited to: increased analysis of microbial populations from different feedstocks and preparation methods of vermicompost and vermicompost teas, their impact on citrus fruit quality and mature trees, impacts of vermicompost applications on different citrus rootstock-scion combinations, enhancing vermicompost methods by combining other beneficial

microorganisms into applications, and of course, specific studies on the effects of vermicompost applications on ACP.

Culture and analysis of compost tea microorganisms and densities between brewing methods as demonstrated by Fritz, Franke-Whittle, Haindl, Insam, & Braun (2012) would provide very valuable insight into the most effective methods for increasing the potency of beneficial microorganisms in aerated vermicompost liquid solutions. This would lead to improved methodology for vermicompost brews and a more thorough identification of the particular microorganisms that are most beneficial in applications.

Experiments on mature citrus trees would be very significant because fruit quality is another major area of importance for citrus growers concerned with producing high quality fruit for the fresh citrus market. Several studies have indicated that vermicompost applications have led to an increase in nutrient status and overall quality in various annual crops, which points to similar possibilities with respect to citrus fruits (Lazcano, Revilla, Malvar, Dominguez, & Ana Malvar, 2011; Fritz et al., 2012). Further study in assessing the ways in which vermicompost applications can improve factors such as sugar and nutrient content, external fruit appearance free of scars and blemishes, fruit size, and overall yield would provide invaluable insight. In order to evaluate these important aspects of citrus fruit quality, it would be beneficial to design a study that determines citrus fruit quality by methods such as total soluble solids, acid ratio via titration, etc. (Continella et al., 2018), as well as the leaf nutrient analysis to examine overall tree health (Sharma, Dubey, Awasthi, & Kaur, 2016).

As with many other studies, vermicompost applications can be used in combination with other known beneficial bioinoculants, such as plant growth promoting

rhizobacteria in very promising ways (Singh, Divya, Awasthi, & Kalra, 2012; Utkhede, & Koch, 2004). As previously mentioned, there have also been successful efforts to increase the humic acid content in vermicompost with novel strains of trichoderma fungal inoculations (Maji, Singh, Wasnik, Chanotiya, & Kalra, 2015). Determining the best methods of enhancing the already nutrient dense and microbially diverse vermicompost is still an area of great potential.

Different rootstock-scion combinations are also an area of study that would be greatly beneficial to study in conjunction with response to aerated vermicompost tea and the rate of growth of young citrus nursery trees. It is well known that different rootstock and scion combinations can have a wide range of different qualities, and it would be very useful to determine whether or not certain rootstocks, scions, or rootstock-scion combinations are more receptive to positive effects from the aerated vermicompost tea treatment than others (Sharma, Dubey, Awasthi, & Kaur, 2016).

Water quality was also undoubtedly a very considerable factor throughout the course of this experiment. Further studies on vermicompost aqueous solutions should consider the major impact that water quality can have on the end result of the product. High amounts of pollutants and other compounds commonly found in irrigation water can have unforeseen impacts on the development of beneficial microorganisms. Furthermore, the chlorine used in municipal water treatment to kill pathogens can negatively affect the beneficial microorganisms that are present in aqueous vermicompost solutions. Due to the fact that many water sources, especially in urban settings, are treated with chlorine, this is also something that needs to be studied in greater detail. Useful studies might include developing specific negative-effects thresholds for chlorine content in aqueous

vermicompost solutions by quantifying microbial populations with varying concentrations of chlorine, as well as other pollutants. Further studies on cost-effective removal of chlorine and other contaminants would be necessary as well for practical agricultural and horticultural applications, since the use of aqueous vermicompost solutions would need to be economically feasible in order to be adopted on a wide enough scale to offset the current environmentally detrimental practices.

Finally, although the results of this study were inconclusive, research specifically focused on ACP populations and their response to aerated aqueous vermicompost solutions applied to citrus trees in the field would be most beneficial for determining pest resistance to this potentially devastating disease vector.

REFERENCES

Aira, Olcina, Pérez-Losada, & Domínguez. (2016). Characterization of the bacterial communities of casts from Eisenia andrei fed with different substrates. *Applied Soil Ecology, 98*, 103-111.

Ansari, A., & Sukhraj, K. (2010). Effect of vermiwash and vermicompost on soil parameters and productivity of okra (Abelmoschus esculentus) in Guyana. *African Journal Of Agricultural Research, 5*(14), 1794-1798.

Arancon, Edwards, Atiyeh, & Metzger. (2004). Effects of vermicomposts produced from food waste on the growth and yields of greenhouse peppers. *Bioresource Technology, 93*(2), 139-144.

Arancon, Edwards, Babenko, Cannon, Galvis, & Metzger. (2008). Influences of vermicomposts, produced by earthworms and microorganisms from cattle manure, food waste and paper waste, on the germination, growth and flowering of petunias in the greenhouse. *Applied Soil Ecology, 39*(1), 91-99.

Arancon, N. Q., Edwards, C. A., Bierman, P., Metzger, J. D., & Lucht, C. (2005a). Effects of vermicomposts produced from cattle manure, food waste and paper waste on the growth and yield of peppers in the field. *Pedobiologia*, *49*(4), 297-306.

Arancon, Edwards, Lee, & Byrne. (2006). Effects of humic acids from vermicomposts on plant growth. *European Journal of Soil Biology, 42*, S65-S69.

Arancon, N. Q., Galvis, P. A., & Edwards, C. A. (2005b). Suppression of insect pest populations and damage to plants by vermicomposts. Bioresource Technology, 96(10), 1137-1142.

Atiyeh, R. M., Domínguez, J., Subler, S., & Edwards, C. A. (2000a). Changes in biochemical properties of cow manure during processing by earthworms (Eisenia andrei, Bouché) and the effects on seedling growth. *Pedobiologia*, *44*(6), 709-724.

Atiyeh, R., Edwards, C., Subler, S., & Metzger, J. (2000b). Earthworm-Processed Organic Wastes as Components Of Horticultural Potting Media for Growing Marigold and Vegetable Seedlings. *Compost Science & Utilization, 8*(3), 215-223.

Atiyeh, R. M., Lee, S., Edwards, C. A., Arancon, N. Q., & Metzger, J. D. (2002). The influence of humic acids derived from earthworm-processed organic wastes on plant growth. *Bioresource technology*, *84*(1), 7-14.

Babcock, B. (2018). Economic impact of California's citrus industry. *Citrograph*, *9*, 36-39.

Biradar, A.P., Sunita, N.D., Teggel, R.G., Devarandavadgi, S.B. (1998). Effect of vermicompost on the incidence of subabul psyllid. Insect-Envi., 4 (2) (1998), pp. 55-56.

Brar, & Spann. (2014). Photoperiodic phytochrome-mediated vegetative growth responses of container-grown citrus nursery trees. *Scientia Horticulturae, 176*, 112-119.

Canellas, L., Olivares, F., Okorokova-Facanha, A., & Facanha, A. (2002). Humic acids isolated from earthworm compost enhance root elongation, lateral root emergence, and plasma membrane H -ATPase activity in maize roots. *Plant Physiology, 130*(4), 1951-1957.

CDFA. (2019a). California Department of Food and Agriculture. Retrieved from https://www.cdfa.ca.gov/plant/acp/regulation.html

CDFA. (2019b). California Department of Food and Agriculture. Retrieved from https://www.cdfa.ca.gov/plant/hlb/regulation.html

Continella, Pannitteri, La Malfa, Legua, Distefano, Nicolosi, & Gentile. (2018). Influence of different rootstocks on yield precocity and fruit quality of 'Tarocco Scirè' pigmented sweet orange. *Scientia Horticulturae, 230*, 62-67.

Dees, P., & Ghiorse, W. (2001). Microbial diversity in hot synthetic compost as revealed by PCR-amplified rRNA sequences from cultivated isolates and extracted DNA. *FEMS Microbiology Ecology, 35*(2), 207-216.

Doan, T. T., Ngo, P. T., Rumpel, C., Van Nguyen, B., & Jouquet, P. (2013). Interactions between compost, vermicompost and earthworms influence plant growth and yield: A one-year greenhouse experiment. *Scientia Horticulturae*, *160*, 148-154.

Dreistadt, S., & University of California Integrated Pest Management Program. (2012). *Integrated pest management for citrus.* (Third ed., Publication (University of California (System). Division of Agriculture and Natural Resources); 3303). Richmond, CA: University of California, Statewide Integrated Pest Management Program, Agriculture and Natural Resources.

Eastman, B. R., Kane, P. N., Edwards, C. A., Trytek, L., Gunadi, B., Stermer, A. L., & Mobley, J. R. (2001). The effectiveness of vermiculture in human pathogen reduction for USEPA biosolids stabilization. *Compost Science & Utilization*, *9*(1), 38-49.

Edwards, C. A. (Ed.). (2004). *Earthworm ecology*. CRC press.

Edwards, Arancon, Vasko-Bennett, Askar, Keeney, & Little. (2010). Suppression of green peach aphid (Myzus persicae) (Sulz.), citrus mealybug (Planococcus citri) (Risso), and two spotted spider mite (Tetranychus urticae) (Koch.) attacks on tomatoes and cucumbers by aqueous extracts from vermicomposts. Crop Protection, 29(1), 80-93.

Edwards, C. A., & Fletcher, K. E. (1988). Interactions between earthworms and microorganisms in organic-matter breakdown. *Agriculture, Ecosystems & Environment*, *24*(1-3), 235-247.

Ferguson, L., Grafton-Cardwell, E., & University of California. Division of Agriculture Natural Resources. (2014). *Citrus production manual* (First ed., Publication (University of California (System). Division of Agriculture and Natural Resources); 3539).

Ferrarezi, R. S. (2019). [HS1333] CNPG Chapter 8) Stock plant and Tree production: b) Irrigation and Fertilization.

Fracchia, L., Dohrmann, A., Martinotti, B., & Tebbe, M. (2006). Bacterial diversity in a finished compost and vermicompost: Differences revealed by cultivation-independent analyses of PCR-amplified 16S rRNA genes. *Applied Microbiology and Biotechnology, 71*(6), 942-952.

Frederickson, Butt, Morris, & Daniel. (1997). Combining vermiculture with traditional green waste composting systems. *Soil Biology and Biochemistry, 29*(3), 725-730.

Fritz, J. I., Franke-Whittle, I. H., Haindl, S., Insam, H., & Braun, R. (2012). Microbiological community analysis of vermicompost tea and its influence on the

growth of vegetables and cereals. *Canadian journal of microbiology*, *58*(7), 836-847.

Grafton-Cardwell, E. E., Stelinski, L. L., & Stansly, P. A. (2013). Biology and management of Asian citrus psyllid, vector of the huanglongbing pathogens. *Annual Review of Entomology*, *58*, 413-432.

Guazzelli, L., Davies, F. S., Ferguson, J. J., & Castle, W. S. (1995). Nitrogen Nutrition and Growth of Hamlin Orange Nursery Trees on Swingle Citrumelo Rootstock. *HortTechnology*, *5*(2), 147-149.

Ingram, D. T., & Millner, P. D. (2007). Factors affecting compost tea as a potential source of Escherichia coli and Salmonella on fresh produce. *Journal of food protection*, *70*(4), 828-834.

Islam, Yaseen, Traversa, Ben Kheder, Brunetti, & Cocozza. (2016). Effects of the main extraction parameters on chemical and microbial characteristics of compost tea. *Waste Management, 52*, 62-68.

Jack, A. L., & Thies, J. E. (2006). Compost and vermicompost as amendments promoting soil health. *Biological approaches to sustainable soil systems*, 453-466.

Jeyabal, & Kuppuswamy. (2001). Recycling of organic wastes for the production of vermicompost and its response in rice–legume cropping system and soil fertility. *European Journal of Agronomy, 15*(3), 153-170.

Joshi, R., Singh, J., & Vig, A. P. (2015). Vermicompost as an effective organic fertilizer and biocontrol agent: effect on growth, yield and quality of plants. Reviews in Environmental Science and Bio/Technology, 14(1), 137-159.

Kim, M. J., Shim, C. K., Kim, Y. K., Hong, S. J., Park, J. H., Han, E. J., ... & Kim, S. C. (2015). Effect of aerated compost tea on the growth promotion of lettuce, soybean, and sweet corn in organic cultivation. *The plant pathology journal*, *31*(3), 259.

Kjellin, M., Johansson, I., & Johansson, Ingegärd. (2010). *Surfactants from renewable resources* (Wiley Series in Renewable Resource). Chichester, West Sussex: Wiley.

Koné, Dionne, Tweddell, Antoun, & Avis. (2010). Suppressive effect of non-aerated compost teas on foliar fungal pathogens of tomato. *Biological Control, 52*(2), 167-173.

Lazcano, C., Revilla, P., Malvar, R., Dominguez, J., & Ana Malvar, C. (2011). Yield and fruit quality of four sweet corn hybrids (Zea mays) under conventional and integrated fertilization with vermicompost. *Journal Of The Science Of Food And Agriculture, 91*(7), 1244-1253.

Lazcano, C., Gómez-Brandón, M., & Domínguez, J. (2008). Comparison of the effectiveness of composting and vermicomposting for the biological stabilization of cattle manure. *Chemosphere*, *72*(7), 1013-1019.

Maji, Misra, Singh, & Kalra. (2017). Humic acid rich vermicompost promotes plant growth by improving microbial community structure of soil as well as root nodulation and mycorrhizal colonization in the roots of Pisum sativum. *Applied Soil Ecology, 110*, 97-108.

Maji, D., Singh, M., Wasnik, K., Chanotiya, C., & Kalra, A. (2015). The role of a novel fungal strain Trichoderma atroviride RVF3 in improving humic acid content in

mature compost and vermicompost via ligninolytic and celluloxylanolytic activities. *Journal of Applied Microbiology, 119*(6), 1584-1596.

McQuilken, M., Whipps, P., & Lynch, J. (1994). Effects of water extracts of a composted manure-straw mixture on the plant pathogen Botrytis cinerea. *World Journal of Microbiology and Biotechnology, 10*(1), 20-26.

Mulvaney, R. L., Khan, S. A., & Ellsworth, T. R. (2009). Synthetic nitrogen fertilizers deplete soil nitrogen: a global dilemma for sustainable cereal production. *Journal of environmental quality*, *38*(6), 2295-2314.

Munk, D., & Hartz, T. (2017). Groundwater Protection in California: Nitrogen Planning and Reporting. *Western Nutrient Management Conference. Vol.12.* Reno, NV.

Noble, R., & Roberts, S. J. (2004). Eradication of plant pathogens and nematodes during composting: a review. *Plant pathology*, *53*(5), 548-568.

Palmer, A. K., Evans, K. J., & Metcalf, D. A. (2010). Characters of aerated compost tea from immature compost that limit colonization of bean leaflets by Botrytis cinerea. *Journal of applied microbiology*, *109*(5), 1619-1631.

Pant, A., Radovich, T., Hue, N., & Arancon, N. (2011). Effects of Vermicompost Tea (Aqueous Extract) on Pak Choi Yield, Quality, and on Soil Biological Properties. *Compost Science & Utilization, 19*(4), 279-292.

Pathma, J., & Sakthivel, N. (2012). Microbial diversity of vermicompost bacteria that exhibit useful agricultural traits and waste management potential. *SpringerPlus*, *1*(1), 26.

Pieterse, C. M., van Pelt, J. A., Verhagen, B. W., Ton, J., van Wees, A. C. M., Léon-Kloosterziel, K. M., & Van Loon, L. C. (2003). Induced systemic resistance by plant growth-promoting rhizobacteria. *Symbiosis*, *35*(1-3), 39-54.

Pimentel, D., & Edwards, C. (1982). Pesticides and Ecosystems. *BioScience, 32*(7), 595-600.

Plavšin, I., Velki, M., Ečimović, S., Vrandečić, K., & Ćosić, J. (2017). Inhibitory effect of earthworm coelomic fluid on growth of the plant parasitic fungus Fusarium oxysporum. *European journal of soil biology*, *78*, 1-6.

Rajpal, A., Bhargava, R., Chopra, A. K., & Kumar, T. (2014). Vermistabilization and nutrient enhancement of anaerobic digestate through earthworm species Perionyx excavatus and Perionyx sansibaricus. *Journal of Material Cycles and Waste Management*, *16*(2), 219-226.

Schaffer, Peña, Colls, & Hunsberger. (1997). Citrus leafminer (Lepidoptera: Gracillariidae) in lime: Assessment of leaf damage and effects on photosynthesis. *Crop Protection, 16*(4), 337-343.

Scheuerell, S., & Mahaffee, W. (2002). Compost Tea: Principles and Prospects For Plant Disease Control. *Compost Science & Utilization, 10*(4), 313-338.

Scheuerell, S. J., & Mahaffee, W. F. (2004). Compost tea as a container medium drench for suppressing seedling damping-off caused by Pythium ultimum. *Phytopathology*, *94*(11), 1156-1163.

Sharma, Dubey, Awasthi, & Kaur. (2016). Growth, yield, fruit quality and leaf nutrient status of grapefruit (Citrus paradisi Macf.): Variation from rootstocks. *Scientia Horticulturae, 210*, 41-48.

Singh, R., Divya, S., Awasthi, A., & Kalra, A. (2012). Technology for efficient and successful delivery of vermicompost colonized bioinoculants in Pogostemon cablin (patchouli) Benth. *World Journal of Microbiology and Biotechnology, 28*(1), 323-33.

Spreen, T. H., Baldwin, J. P., & Futch, S. H. (2014). An economic assessment of the impact of Huanglongbing on citrus tree plantings in Florida. *HortScience*, *49*(8), 1052-1055.

Stansly, P., & Kostyk, B. (2018). Foliar Sprays of Cyclaniliprole and Abamectin to Control Asian Citrus Psyllid and Citrus Leafminer on Orange: Fall, 2016. *Arthropod Management Tests, 43*(1), Arthropod Management Tests, 2018, Vol. 43(1).

Suharsi, T. K., & Sari, A. D. P. (2014). Pertumbuhan mata tunas jeruk keprok (Citrus nobilis) hasil okulasi pada berbagai media tanam dan umur batang bawah rough lemon (C. jambhiri). *Jurnal Ilmu Pertanian Indonesia*, *18*(2), 97-101.

Timmer, L. W., Graham, J. H., & Zitko, S. E. (1998). Metalaxyl-resistant isolates of Phytophthora nicotianae: occurrence, sensitivity, and competitive parasitic ability on citrus. *Plant Disease*, *82*(2), 254-261.

Tognetti, C., Laos, F., Mazzarino, M. J., & Hernandez, M. T. (2005). Composting vs. vermicomposting: a comparison of end product quality. *Compost Science & Utilization*, *13*(1), 6-13.

USDA releases state EQIP allocations. (2002). *Southwest Farm Press*, 13. Retrieved from http://proxy.library.cpp.edu/login?url=https://search-proquest-com.proxy.library.cpp.edu/docview/199446217?accountid=10357 SUDA

USDA and CDFA host joint summit on healthy soils. (2016, Dec 21). *PR Newswire* Retrieved from http://proxy.library.cpp.edu/login?url=https://search.proquest.com/docview/1850763854?accountid=10357

USDA. (2019a). United States Department of Agriculture National Agricultural Statistics Service. Retrieved from https://quickstats.nass.usda.gov/results/8FFF3170-A639-3A7E-AE81-A1EFB167CC56

USDA. (2019b). United States Department of Agriculture National Agricultural Statistics Service. Retrieved from https://quickstats.nass.usda.gov/results/B9A8AFED-DC20- 3654-88F4-ADD4B9BC4982

USDA. (2019c). United States Department of Agriculture National Agricultural Statistic Service. Retrieved from https://quickstats.nass.usda.gov/results/3CFDA473-1B34-378E-8C5E-5D47C1FAA057

Utkhede, R., & Koch, C. (2004). Biological treatments to control bacterial canker of greenhouse tomatoes. *BioControl, 49*(3), 305-313.

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

Yadav, R. K., Jain, M. C., & Jhakar, R. P. (2012). Effect of media on growth and development of acid lime (Citrus aurantifolia Swingle) seedling with or without Azotobacter. *African Journal of Agricultural Research*, *7*(48), 6421-6426.

Yadav, K. D., Tare, V., & Ahammed, M. M. (2010). Vermicomposting of source-separated human faeces for nutrient recycling. *Waste Management*, *30*(1), 50-56.

Yasir, Aslam, Kim, Lee, Jeon, & Chung. (2009). Bacterial community composition and chitinase gene diversity of vermicompost with antifungal activity. *Bioresource Technology, 100*(19), 4396-4403.

Zhang, H., Tan, S., Wong, W., Ng, C., Teo, C., Ge, L., . . . Yong, J. (2014). Mass spectrometric evidence for the occurrence of plant growth promoting cytokinins in vermicompost tea. *Biology And Fertility Of Soils, 50*(2), 401-403.

APPENDIX A

Table 1: Final dry leaf biomass (g) from potted citrus trees receiving one of four aqueous vermicompost solution treatments monthly, from July 2018 to July 2019.

	Leaf Mass*										
	Replications										
Treaments	1	2	3	4	5	6	7	8	9	10	**Mean**
Control	17.6	25.7	37.7	45.2	44.3	27.2	43.2	39.1	60	50.2	39.02 A**
Soil	43.5	51.3	29.6	20.6	44.3	35.4	54.3	38.2	52.8	43.7	41.37 A
Foliar	44	49.3	44	49	49.9	56.9	54.8	51.2	54.9	44.2	49.82 A
Soil + Foliar	43	40.7	55	44.4	46.9	26.2	47.2	18.5	35.3	54.8	41.2 A
Summary of ANOVA Effects (Treatment)											*P*= 0.1172

*Leaf mass measured in grams.

**Means within the same column followed by the same letter are not significantly different; Fisher's LSD test, *P*=0.05.

Table 2: Final dry shoot biomass (g) from potted citrus trees receiving one of four aqueous vermicompost solution treatments monthly, from July 2018 to July 2019.

	Shoot Mass*										
	Replications										
Treaments	1	2	3	4	5	6	7	8	9	10	**Mean**
Control	44.5	62.6	70	69.9	74.5	52.4	73.7	75.6	83.1	67.4	67.37 A**
Soil	87.4	86.9	60.2	57.9	68.6	78	86.5	73.3	74	76.1	74.89 A
Foliar	77.3	81.9	51.4	91.2	78.9	77.6	88.8	95	76.9	78.3	79.73 A
Soil + Foliar	75	81.4	86.6	65.9	79.9	52.6	76	38.1	65	70.9	69.14 A
Summary of ANOVA Effects (Treatment)											*P*= 0.1141

*Shoot mass measured in grams.

**Means within the same column followed by the same letter are not significantly different; Fisher's LSD test, *P*=0.05.

Table 3: Final dry root biomass (g) from potted citrus trees receiving one of four aqueous vermicompost solution treatments monthly, from July 2018 to July 2019.

	Root Mass*										
	Replications										
Treaments	1	2	3	4	5	6	7	8	9	10	**Mean**
Control	41.3	53.3	85	88.9	78.7	39.8	101.6	122.9	97.6	114.4	82.35 A**
Soil	148.2	92.3	54.9	42.2	117.8	117.6	76.4	98.9	60	105.4	91.37 A
Foliar	94.4	196.7	85.7	115.6	152.8	120.8	108	133.5	114.9	136.5	125.89 A
Soil + Foliar	59.1	72.4	166.8	91.3	182.4	38.7	104	28.2	80.6	73.2	89.67 A
Summary of ANOVA Effects (Treatment)											*P*= 0.0562

*Root mass measured in grams.

**Means within the same column followed by the same letter are not significantly different; Fisher's LSD test, *P*=0.05.

Table 4: Final trunk diameter (mm) from potted citrus trees receiving one of four aqueous vermicompost solution treatments monthly, from July 2018 to July 2019.

	Trunk Diameter*										
	Replications										
Treaments	1	2	3	4	5	6	7	8	9	10	**Mean**
Control	13.5	16.1	17.2	18.1	16.7	15	19.4	17.4	17.7	17.5	16.86 A**
Soil	18.7	19.4	15.9	16.1	17.9	22.8	18.3	17.3	18.5	16.8	18.17 A
Foliar	17.5	18.2	14.8	19.1	19.3	18	19.6	19.4	18.6	18.6	18.31 A
Soil + Foliar	17.5	19.5	19.5	18.6	17.8	16	18.1	13.1	17.3	17.9	17.53 A
Summary of ANOVA Effects (Treatment)											*P*= 0.2436

*Trunk diameter measured in millimeters.

**Means within the same column followed by the same letter are not significantly different; Fisher's LSD test, *P*=0.05.

Table 5: Mean monthly Asian citrus psyllid populations per tree from February to July 2019 on potted citrus trees receiving one of four aqueous vermicompost solution treatments monthly, from July 2018 to July 2019.

	Asian Citrus Psyllids*						
	Month						
Treatment	February	March	April	May	June	July	**Mean**
Control	0.3 a	0.1 a	0 a	0.1 a	0 a	0.1 a	0.12 A**
Soil	0.1 a	0 a	0.1 a	0.1 a	0 a	0.1 a	0.08 A
Foliar	0.2 a	0.3 a	0 a	0 a	0 a	0 a	0.1 A
Soil + Foliar	0.3 a	0.3 a	0.2 a	0 a	0.1 a	0 a	0.18 A
Mean	0.225 a	0.175 ab	0.075 bc	0.050 bc	0.025 c	0.050 bc	
	Summary of ANOVA Effects *(P)*						
Treatment	0.7685	0.2091	0.2829	0.5780	0.4040	0.5780	0.5935
Treatment x Replication	-	-	-	-	-	-	0.1172
Month	-	-	-	-	-	-	0.0168
Treatment x Month	-	-	-	-	-	-	0.5376

*Mean ACP count per tree based on 10 replications.
**Means within the same row or column followed by the same letter are not significantly different; Fisher's LSD test, *P*=0.05.

Table 6: Mean monthly thrips populations per tree from March to July 2019 on potted citrus trees receiving one of four aqueous vermicompost solution treatments monthly, from July 2018 to July 2019.

	Thrips*						
	Month						
Treatment	February	March	April	May	June	July	**Mean**
Control	-	0.1 a	0.1 a	0.8 ab	1.6 a	1 a	0.72 A**
Soil	-	0 a	0.2 a	0.4 ab	1.3 a	0.5 a	0.48 A
Foliar	-	0 a	0 a	1 a	0.7 a	0.5 a	0.44 A
Soil + Foliar	-	0 a	0.1 a	0.2 b	0.7 a	1.1 a	0.42 A
Mean	**-**	0.025 b	0.1 b	0.6 a	1.075 a	0.775 a	
	Summary of ANOVA Effects *(P)*						
Treatment	-	0.404	0.5548	0.1513	0.59	0.6336	0.4604
Treatment x Replication	-	-	-	-	-	-	0.5636
Month	-	-	-	-	-	-	<0.0001
Treatment x Month	-	-	-	-	-	-	0.6940

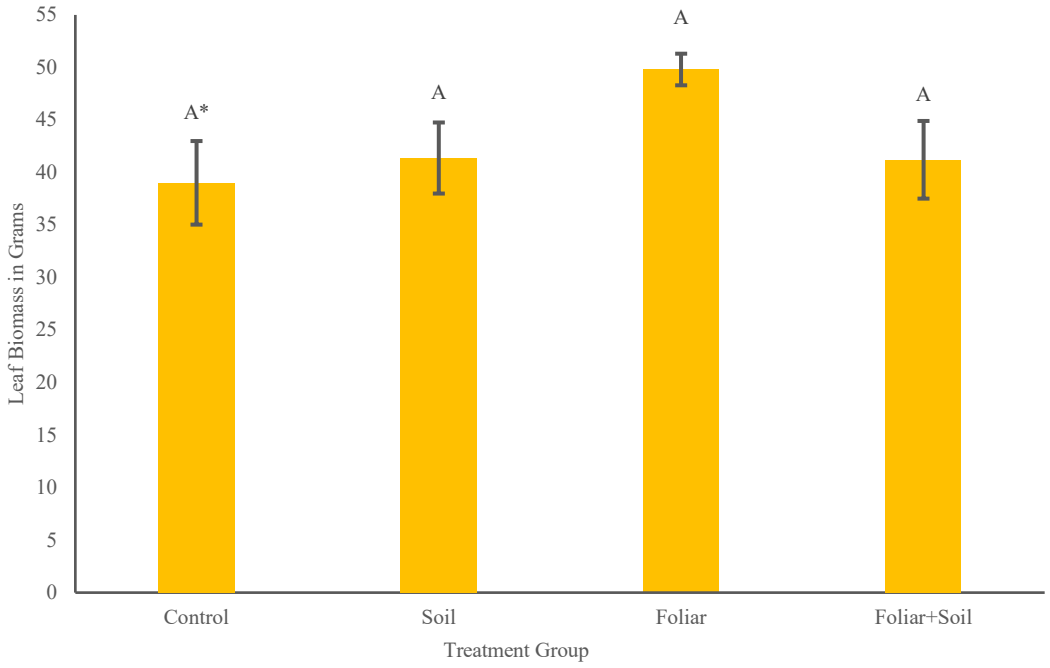
*Mean thrips count per tree based on 10 replications.
**Means within the same row or column followed by the same letter are not significantly different; Fisher's LSD test, *P*=0.05.

Table 7: Mean monthly leafminer populations per tree from May to July 2019 on potted citrus trees receiving one of four aqueous vermicompost solution treatments monthly, from July 2018 to July 2019.

	Leafminer*						
	Month						
Treatment	February	March	April	May	June	July	**Mean**
Control	-	-	-	0.7 a	0.5 a	0.7 a	0.6333 A**
Soil	-	-	-	0.1 b	0 a	0.4 a	0.1667 B
Foliar	-	-	-	0 b	0.4 a	0.4 a	0.2667 B
Soil + Foliar	-	-	-	0.1 b	0.5 a	0.2 a	0.2667 B
Mean	**-**	**-**	**-**	0.225 a	0.35 a	0.425 a	
	Summary of ANOVA Effects *(P)*						
Treatment	-	-	-	0.0016	0.2815	0.3802	0.0204
Treatment x Replication	-	-	-	-	-	-	0.3330
Month	-	-	-	-	-	-	0.2837
Treatment x Month	-	-	-	-	-	-	0.3281

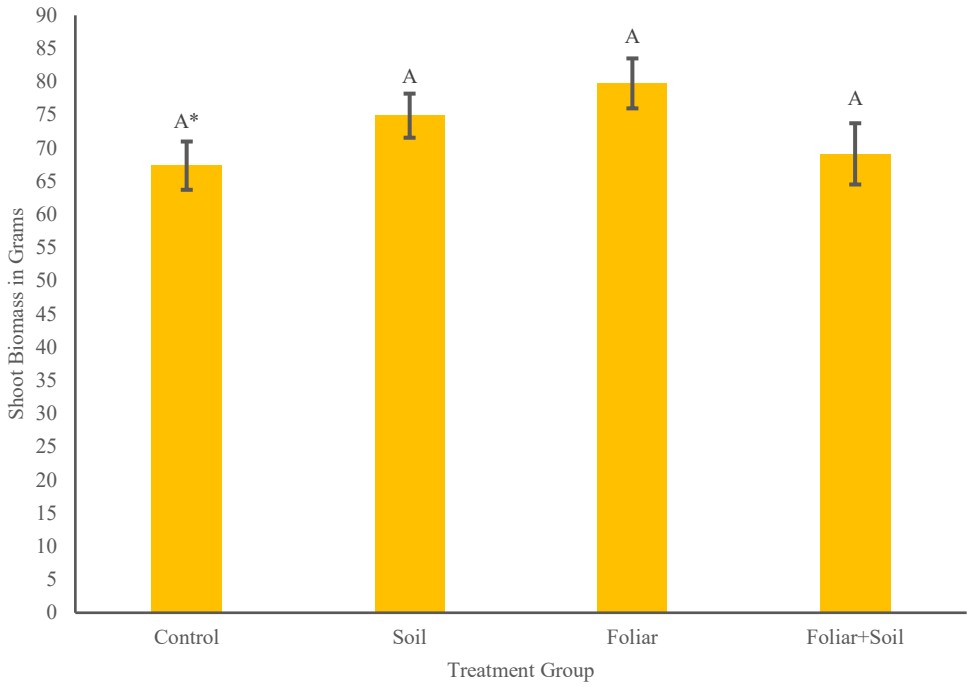
*Mean leafminer count per tree based on 10 replications.
**Means within the same row or column followed by the same letter are not significantly different; Fisher's LSD test, *P*=0.05.

APPENDIX B



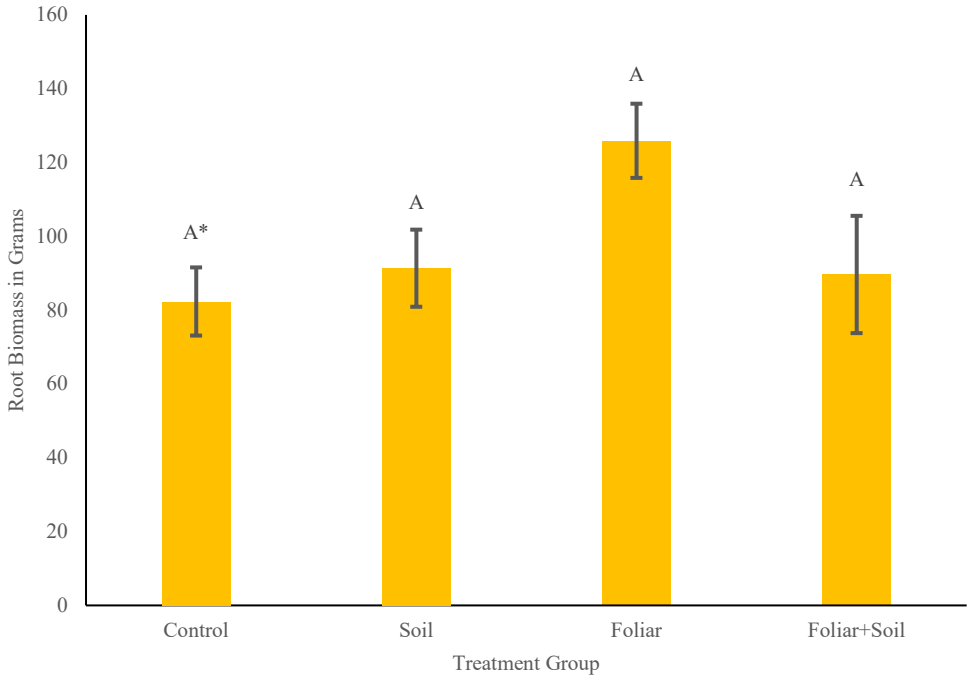
*Means followed by the same letter are not statistically significant, Fisher's LSD test, *P*=0.05.
Error bars indicate standard error of the mean.

Figure 1: Final dry leaf biomass (g) from potted citrus trees receiving one of four aqueous vermicompost solution treatments monthly, from July 2018 to July 2019.



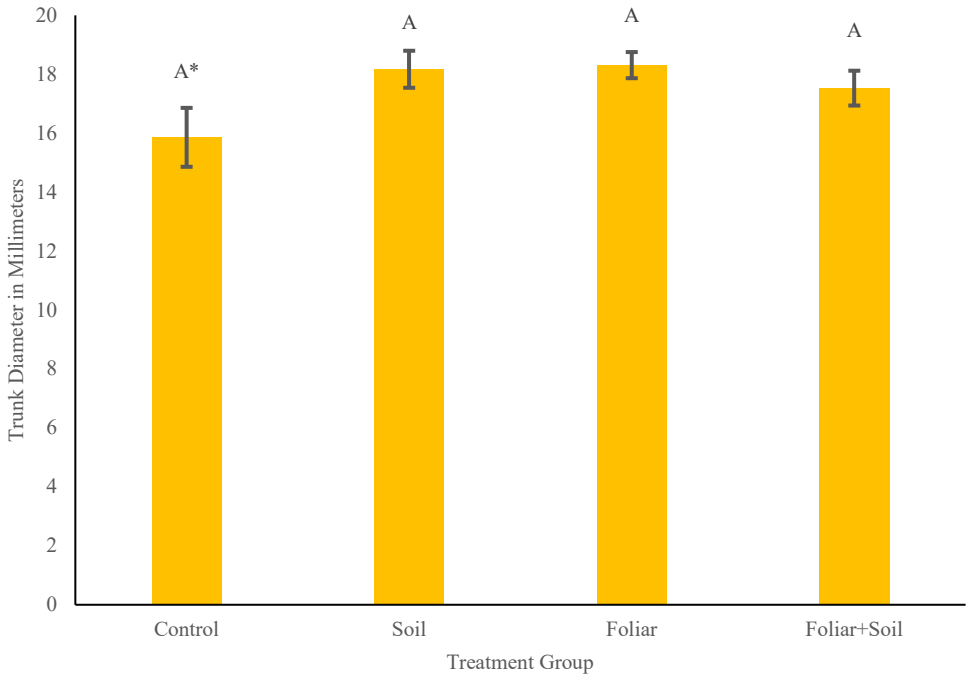
*Means followed by the same letter are not statistically significant, Fisher's LSD test, *P*=0.05.
Error bars indicate standard error of the mean.

Figure 2: Mean shoot biomass in grams.



*Means followed by the same letter are not statistically significant, Fisher's LSD test, *P*=0.05.
Error bars indicate standard error of the mean.

Figure 3: Mean root biomass in grams.



*Means followed by the same letter are not statistically significant, Fisher's LSD test, *P*=0.05.
Error bars indicate standard error of the mean.

Figure 4:Mean trunk diameter in millimeters.

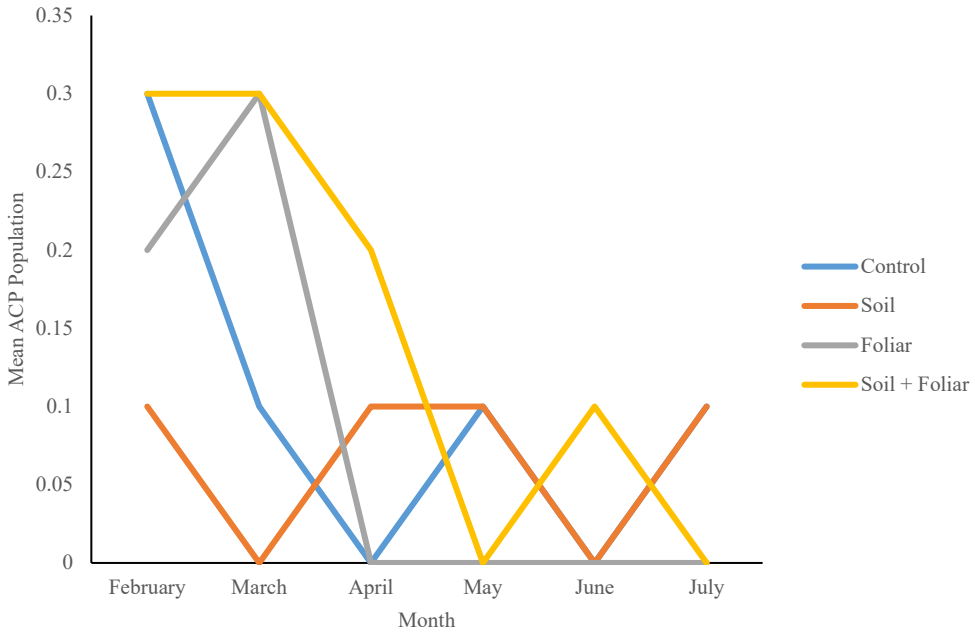


Figure 5: Mean ACP populations.

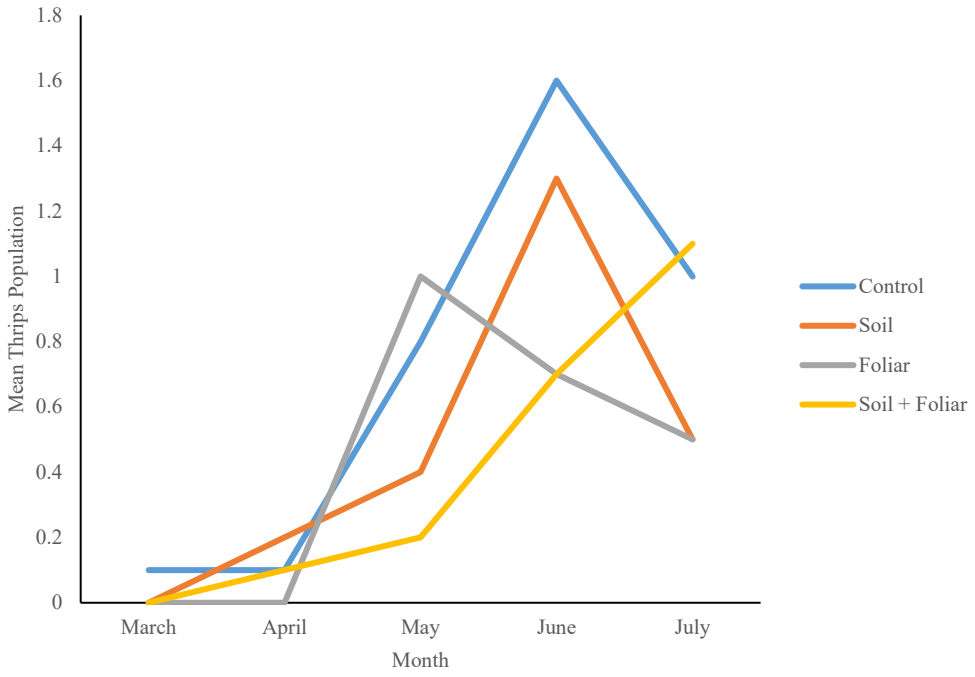


Figure 6: Mean thrips populations.

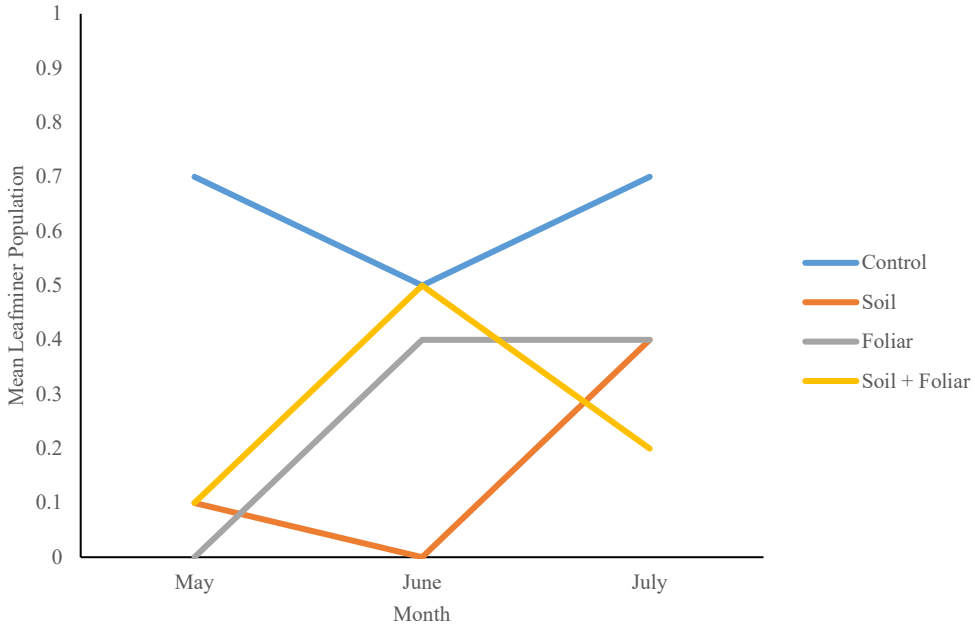


Figure 7: Mean leafminer populations.