INVESTIGATING *OSMIA LIGNARIA* AS STRAWBERRY POLLINATORS FOR URBAN VERTICAL FARMS

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May 4th, 2020

This paper represents my own work in accordance with University regulations.

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1. Abstract

Current population projections and the continued use of unsustainable agricultural practices point to a real possibility of a global food shortage. Vertical farming offers one potential solution to these issues by increasing food production without increasing land use. However, vertical farm development is restrained by high labor costs among other issues. This project explores the use of insect pollination in vertical farming to reduce these labor costs and produce higher quality crops. Specifically, we investigated the pollination ability of the solitary bee, *Osmia lignaria*, in a vertical farm growing strawberries. By setting up three small vertical farms, we examined the differences in fruit amount, size, quality and overall plant health in the presence and absence of *O. lignaria*. We further analyzed whether UV spectrum light and room temperature affected bee health and strawberry production. Our results indicate that *O. lignaria* can, indeed, pollinate strawberries in a vertical farm. Both strawberry production and bee health were better in cooler temperatures, and bee mortality rates were lower in the presence of UV light. However, *O. lignaria's* unique life cycle may cause complications for their year-round use. Regardless, our findings suggest solitary bees could be a useful resource to reduce vertical farm costs, but they also highlight the need for further research to develop vertical farming.

2) Introduction

2.1) The Need for Novel Food Production Strategies

Global agriculture is faced with an urgent need to adapt. The ways we produce and consume our food will need to change. The United Nations predicts the world's population to increase from 7 billion measured in 2010 to over 9.5 billion in 2050 (United Nations, 2015). With this spike, global food systems will need to confront at least a fifty percent increase in overall food demand by 2050 (FAO, 2009; Odegard & van der Voet, 2014). As income increases across the developing world, demands for land-intensive products such as meat and dairy are expected to increase by nearly 70% (Lotze-Campen et al., 2006; Searchinger et al., 2018). Meeting, and possibly even changing, these projected food demands will require our utmost attention today.

According to the World Bank in 2016, approximately 37% of global land area is already used for food production (World Bank, 2016). In the United States, this number proves even more staggering as over 50% of land across the contingent 48 US states is classified as either cropland or grassland pasture (Lubowski et al., 2006). With this amount of land use, agriculture is the leading contributor to global deforestation. Agriculture accounts for more than 10 million of the 11.6 million hectares of forest lost each year across the globe (Pimentel et al., 1986). In the face of rapid climate change, one of the most pressing issues caused by deforestation is the loss of carbon sequestration by old growth forests. Scientists are searching for new ways to balance land for storing carbon and land for producing food.

Agriculture's contributions to greenhouse gas emissions (GHG) are vast because of both land use change and operating costs. Agriculture and its associated land use changes accounts for

21%, nearly one-quarter, of global GHG emissions annually (Goldstein, 2018). According to other researchers' calculations, this measure may be unreliable and low when gross land use changes are considered instead of net changes (Searchinger, 2011). Agriculture's current GHG outputs are already alarming. Under the UN's projections, land use for agriculture is expected to increase by 70% if large agricultural and diet shifts are not made by 2050 (Tilman et al., 2011; United Nations, 2015). However, this prediction, too, may be unrealistically low if developed nations continue to increase their demands for bioenergy which causes an even larger demand for crops (Searchinger, 2011).

Coupled with the dilemma of food production, the world finds itself facing another conflict. We are seeing a global decline in the populations of many animal pollinators including bees, butterflies, and birds (FAO, 2018). Honey bees, specifically, are facing rapid population declines, and scientists aren't exactly sure why (Bee Informed Partnership, 2019; Bromenshenk et al., 2010; Pettis & Delaplane, 2010). This issue is highly interconnected with the topic of food production, as $14 billion worth in crops rely on honey bee pollination in the United States alone (Morse & Calderone, 2000). This crisis pushes scientists to not only search for new ways to produce food but also to find new pollinators and methods to protect our food systems' existing ones.

Other important factors, such as human nutrition and food inequity, are at the forefront of this global dilemma. Today, 1 billion people are malnourished (United Nations, 2010). There exists a disconnect between our food systems, the people they are meant to nourish, and the environment our systems harm. We must find ways to increase food production to meet the nourishment needs of a growing population while also respecting the wellbeing of the environment. The world faces a large dilemma to meet this lofty goal, but many novel food production strategies have come to light.

2.2) Vertical Farms – Promises and Problems

In 2010, Dickson Despommier released a book titled *The Vertical Farm: Feeding the World in the 21st Century*. At the time of its release, vertical farms were not a new idea; other countries often refer to them as "Plant Factories" or "Closed Plant Production Systems." However, since the book's release, vertical farms have received large amounts of attention as a potential solution to our food production problems, especially among Western nations. Vertical farms (VF) differ significantly from greenhouses. Although they both are indoor facilities, greenhouses rely on sunlight and are only sometimes supplemented with artificial light. VFs rely solely on artificial (LED) light. This allows for VFs to stack plants in layers and move plants upwards to allow a much greater crop yield per square meter than outdoor agriculture; something greenhouses are unable to do (Goldstein, 2018; Kalantari et al., 2018).

In addition to increasing food amount per unit of land, advocates of VFs claim that they will reduce the environmental footprint of food production. By moving plants indoors and using only hydroponics, VFs do not require herbicides or pesticides and therefore eliminate both production and runoff of the items (Kalantari et al., 2018). In addition, VFs, as a closed system, can use water more efficiently than outdoor agriculture (Beacham et al., 2019; Despommier, 2009). In a closed system, no fertilizer runoff will occur either. Plants can also be grown in shorter periods of time with the use of artificial lights and precise nutrient additions (Kalantari et al., 2018). VFs are often located in urban centers and can grow fresh produce directly where it will be sold and consumed.

However, for all the promises of vertical farms, the growth of this production strategy faces many hurdles to becoming both sustainably and economically viable. First, VFs require a lot of energy. Energy is needed to run the LED lights, temperature control systems, and hydroponic water and nutrient control systems (Yu et al., 2013). This high energy demand is both expensive and currently unsustainable. Because of the potential shade of other buildings and limited space, using solar panels to sustainably provide this energy proves more difficult and expensive than Despommier imagined (Perez, 2014). The second biggest hurdle VFs face is the cost of labor. VFs have not become as automated as traditional farms and therefore require much higher ratios of labor to food produced (Foley, 2018). Our project specifically considers the problem of vertical farm pollination; VFs do not rely on insect pollination, and manual labor is needed to individually hand pollinate each plant in the closed system. The struggle to make profits because of costs associated with energy and labor indicates the need for research on methods to reduce costs in the novel food production strategy of vertical farming.

This project considers the use of insect pollination to alleviate some of the labor needed in VFs. The use of insects to pollinate crops indoors, while scarcely studied in a VF setting, is not entirely new. Two species of bumblebee *(Bombus impatiens* and *Bombus terrestris)* are used in commercial greenhouse tomato production (Ahmad et al., 2015; Vergara & Fonseca-Buendía, 2012; Winter et al., 2006). However, most indoor pollination research has focused on large, social species of bees we traditionally rely on for outdoor agriculture. VFs pose new complications for using social colonies compared to greenhouses which are usually much larger and rely on natural solar light. VFs are likely to be smaller with less open space. Indoor cage experiments of bumblebees show short term ability to pollinate in small spaces by individuals, but do not consider long term use or entire colony health (Thomson et al., 2019). Social bees are unlikely to prosper in a VF as they behave more aggressively and will not react well to the constant disturbance a vertical farm currently requires (Rittschof et al., 2015; Sedivy & Dorn, 2013). In addition, social bees may suffer from disorientation in a small space or abandon a foraging source if rewards decrease (Townsend-Mehler et al., 2011). This project looks elsewhere.

2.3) Strawberries and *Osmia lignaria*

This project focuses on strawberries (*Fragaria x ananassa)* not only because of their beloved taste but also because of their potential to be a successful high returning crop for vertical farms. The United States ranks second in the world for strawberry production and produces over 2,509 million pounds a year (USDA/NASS, 2014). Strawberries are estimated to be worth over $2.2 billion for the United States alone, and this value is expected to continue to grow in the future (Samtani et al., 2019). Land use-wise, strawberries are grown on over 60,000 acres across the United States, and 91% of them are grown in water deficient California (Demchak & Harper, 2005; Samtani et al., 2019). By investigating methods to produce and pollinate strawberries in vertical farms, we investigate a method to increase cost effectiveness of a sustainable way to grow the popular crop.

Strawberry plants, fruits within the family Rosaceae, have hermaphrodite flowers and are considered partially self-fertile. They are, however, not considered completely self-fertilizing (Abrol et al., 2019; McGregor, 1976). This is because, inside the flower, the stamen are positioned in a way that does not allow pollen to accurately drop on all the pistils during self-fertilization. Non-fertilized ovules will not develop fleshy berry tissue which leads to misshapen, smaller, and fewer overall berries (Abrol et al., 2019). Therefore, insect pollination has great positive effects on berry size, quality, and quantity (Herrmann et al., 2019). Currently, honey bees are the main pollinator of strawberry crops around the globe, but as honey bee populations continue to decline scientists turn to native bee species' ability to pollinate strawberries.

Osmia lignaria, also called the "blue orchard bee" or "blue mason bee", is one species of bee that may fit the bill. *O. lignaria* has recently garnered attention as an alternative, native pollinator for many crops such as pears, plums, apples, and cherries (Pinilla-Gallego & Isaacs, 2018). In addition to the orchard crops they are known to fertilize, a study by Horth and Campbell in 2018 indicated that *O. lignaria* can also successfully pollinate strawberry plants. This study displayed that strawberries grown in fields with *O. lignaria* nests had a higher growth rate and larger berry volume (Horth & Campbell, 2018). Native bee species, such as *O. lignaria*, can also pollinate crops more effectively than honey bees (Garibaldi et al., 2013; Pinilla-Gallego & Isaacs, 2018). Our project intends to confirm the ability of *O. lignaria* to pollinate strawberries and investigate this ability in a vertical farm.

Osmia lignaria are a species of solitary bee native to North America. Because of their solitary nature, *O. lignaria* are not known to be aggressive, and females rarely sting humans, even when they are near their nest (Bosch & Kemp, 2001). Although solitary, *O. lignaria* is a gregarious species, and many females will nest in holes located near each other. They are commonly called "blue mason bees" because they use mud to build nests. In the wild, females find holes in trees or abandoned nests from other animals and build a partitioned nest with mud inside them. For farm use, females happily use cardboard nesting tubes (Bosch & Kemp, 2001). The females collect pollen and nectar to provision each partition of their nest. Besides being a solitary species, their low aggression, tolerance to disturbance, and gregarious nature make them a good candidate for use in an indoor farm.

VFs promise to grow crops year-round as they will be devoid of the seasons that traditional agriculture faces. However, this may cause a challenge with using insect pollination in a VF as many species of bees are highly affected by seasonal differences (Fidalgo & Kleinert, 2007). Certain species of bees align their life cycle with seasons, and it can be difficult to rear bees indoors if this is not considered (Greenberg, 1982). Furthermore, little is known about the use of UV spectrum light by *O. lignaria.* Several species of bees utilize UV sensitivity for foraging, orientation, and communication (Spaethe, 2005). However, Ladurner et al. (2003) indicated no difference in the feeding behaviors of *O. lignaria* when under natural light or artificial lights. This project will continue the investigation on the effects of artificial light and controlled seasonality on *O. lignaria*.

2.4) Our Project

In this study, we investigate the viability of *Osmia lignaria* as strawberry pollinators in a vertical farm. We intend to establish preliminary protocols for the use of this pollinator in a vertical farm, while also continuing the investigation of their pollination prowess in relation to strawberries. In order to do this, we set up three small vertical farm rooms each growing strawberry plants following the methods successful vertical farm startups utilize. We compare plants grown in the presence and absence of *O. lignaria* bees. We focus on the differences in berry size, number, fruit quality, and plant health in addition to *O. lignaria* health. We also analyze how different season conditions, such as vertical farm temperature and hours of light, and UV spectrum presence impacts these indicators. Given the single study indicating *O. lignaria's* ability to pollinate strawberries and previous indoor cage experiments, we hypothesize strawberry plants in the presence of *O. lignaria* will show an increase in berry number and size (Horth & Campbell, 2018; Ladurner et al., 2003). Furthermore, since *O. lignaria* typically emerge from cocoons in April, we expect spring-like VF conditions to be best for their health and pollinating ability (Bosch & Kemp, 2001).

3) Methods

3.1) Study Animals

Recently, as honey bee populations decline, scientists and agriculturalists alike are considering the pollination abilities of other bee species. These investigations include the megachilid bee, *Osmia lignaria*. A previous open field experiment indicated that strawberries grown in proximity to *O. lignaria* nests displayed significantly higher berry growth rate and significantly larger berry volume (Horth & Campbell, 2018). To continue the investigation of *O. lignaria* as strawberry pollinators broadly, in addition to their suitability for a vertical farm system, we examined *O. lignaria* in relation to strawberry plant productiveness in a closed system.

We sourced *Osmia lignaria* cocoons from Deweyville, Utah in May of 2019 (from MasonBeesForSale.com). The containers arrived padded with ice packs to keep the cocoons refrigerated during express shipment (2-day travel time). The cocoons were then stored in a refrigerator set to a wintering temperature of 4 °C (Bosch & Kemp, 2000). Bees released during trial 1 (spring) remained inside the refrigerator for approximately 1 week; those released during trial 2 (summer) remained for approximately 5 weeks.

3.2) Study Crop

For this experiment, we chose an everbearing (day neutral) strawberry variety titled the Cabrillo Strawberry. Because of their ability for repeat fruiting, everbearing strawberry varieties were deemed the most useful for vertical farm use (Lewers et al., 2019). Due to time constraints of the study, we ordered already-germinated plants from Lassen Canyon Nursery in Redding, California in May of 2019. Upon arrival, plants were rinsed of dirt and placed in a 16-liter bucket within a separate vertical farm room until set into individual holders for the experiment. During storage, water was changed every other day and 50 mL of germination solution (50% diluted Hoagland solution) was added every week (P. Gauthier, personal communication, June 2019)

3.3) Experimental Design

In this study, we set up three small vertical farm rooms in two environment simulation chambers at Princeton University. The larger environmental simulation chamber was split into two rooms using insect netting, totaling three separate vertical farm "rooms." Each room contained two shelves, and each shelf held one sixteen-liter bucket of hydroponically growing strawberries under RAXRx® and SRYDRx® LED lights ordered from Fluence Bioengineering Vertical Farming Light company. These lights emit a spectrum ranging from 430 nm to 780 nm and peaks at 450 nm and 600 nm (Fluence Bioengineering). Each light was placed approximately 1 meter above the bucket of plants. To confirm consistent light coverage, we measured light intensity every other day using a LiCor 250 light meter (in µmol s^{-1} m^{-2}).

Each 16-liter bucket grew twenty strawberry plants for a total of 40 plants per treatment room per trial. Each plant was placed in a plastic net pot filled with clay pebbles. The pots were placed in a laser-cut plastic bucket cover with roots in the water; the bucket cover was black to inhibit algae growth in the water below. Strawberries were grown in a base nutrient solution following the Hoagland nutrient guidelines for a 16-liter bucket (Gehrmann, 1985). Specific nutrient breakdown of the solution used can be found in Supplementary Table 1. One Aquaneat® aquarium water pump was added to each bucket to provide oxygen. Every day, we measured the electrical conductivity, a proxy for the concentration of nutrients available in a solution, and the pH of each bucket using a Bluelab Guardian monitor. With these two measurements, nutrients were adjusted accordingly. We maintained pH in each bucket between 5.1-6.9; if pH was too high a few drops of diluted Nitric acid were added, and if pH was too low a few drops of NAOH were added, and pH was measured again. We added approximately 1.5 liters of water to each bucket every other day. The strawberry plants' runners were cut immediately upon notice (P. Gauthier, personal communication, June 2019).

To investigate *O. lignaria* pollination, each of the three rooms was assigned a different treatment. Room 1 acted as the control, and no bees were released in this room. In Room 2, approximately 150 ready-to-hatch *O. lignaria* cocoons were added on day 1. In Room 3, we also added 150 ready-to-hatch *O. lignaria* cocoons. Some species of bees use UV spectrum sensitivity for orientation, foraging, or other behaviors (Spaethe, 2005). To investigate if *O. lignaria* need UV spectrum lighting, in Room 3 we also installed a 100-watt, 380-420 nm spectrum light in addition to the LEDs. Supplementary Figure 1 visually displays this experiment set up. Plant health and berry production was compared between the three treatments. The behavior and health of the bees was compared between the presence and absence of the UV light (Room 2 and 3). Self-pollinated strawberry plants produce the fewest and smallest berries compared to both hand and insect pollinated plants (Wietzke et al., 2018). Therefore, to best see the ability of *O. lignaria* to pollinate strawberries, we did not hand-pollinate any of the treatments. We attached Raspberry Pi (Module V2) cameras to the light above each bucket of plants to provide bird's eye view photos and video of the bees and strawberry plants. Photographs of the experiment set up are available in Supplementary Images.

Two trials were run for 30 days each. To consider the temperature and light conditions best suited for *O. lignaria* pollinated strawberry growth, the trials mimicked different season's conditions. We sourced the bees from Utah, so temperature and lighting hours were based off Utah state averages while keeping strawberry requirements in mind (*Climate Utah—Temperature, rainfall and average*, n.d.; Corbett, 1904). trial 1 mimicked spring conditions; temperature was held between 18 °C to 22 °C, and the lights were on for 14 hours a day. trial 2 mimicked Utah's summer conditions: temperature was set between 22°C to 27 °C; the lights were on for 15 hours a day. In each room, an Apple Homekit Eve Degree® Monitor measured temperature and humidity continuously.

3.4) Plant Measurements

To investigate the effects of *O. lignaria* presence and farm season conditions on strawberry production, ripe berries were picked during the experiment, and on Day 30 all berries, regardless of ripeness, were picked. We utilized four different plant measurements to give a robust illustration of *O. lignaria* and strawberry vertical farm compatibility: (1) Number of berries per plant, (2) Volume of strawberries, (3) Dry matter content of strawberries, (4) Root:shoot ratio.

Previous investigations show that plants in the presence of insect pollination have a higher percentage of fruit set than those without (Goodwin, 2010). Therefore, the number of berries per plant was counted and compared between different treatments.

Past literature indicates that insect pollinated plants produce larger and heavier fruits compared to self-pollinated, so the volume of strawberries is a suitable measure of insect pollination ability (Horth & Campbell, 2018; Wietzke et al., 2018). After picking the berries at the end of the thirty-day trial, we measured the height and width of each berry to the nearest mm. Using these measurements, the conical area formula was used to calculate the volume of each berry (Horth & Campbell, 2018):

$$\text{Fruit Volume} = \pi \times \left(\frac{width}{2}\right)^2 \times \left(\frac{height}{3}\right)$$

The dry matter of a fruit acts as a window to the sugars, proteins, and polyphenols that make up the fruit's composition (Ponder & Hallmann, 2019). To investigate how the different treatments impacted strawberry composition, we measured the fresh weight of each berry immediately after picking. Berries were then labeled by which plant they belonged to and stored in a freezer until drying. We dried the strawberries for 48 hours in a drying oven set to 80 °C. They were immediately removed and weighed again. The fresh weights and dry weights of berries were pooled for each individual plant for simplicity. Using the pooled fresh berry weight per plant and the pooled dry berry weight per plant, dry matter content of each plant's berries was calculated using the following formula:

$$\text{Dry Matter Content} = \left(\frac{Pooled\ Dry\ Weight\ of\ Berries\ per\ plant}{Pooled\ Fresh\ Weight\ of\ Berries\ per\ plant} \right) \times 100$$

In addition, the ratio of a plant's root weight to its shoot weight is a useful indicator of its uptake and allocation of resources such as light, CO2, and minerals (Rogers et al., 1995). Therefore, to consider the effects of the treatments on the entire plant, the root:shoot ratio, the dry weight of root biomass divided by the dry weight of shoot biomass, was measured (Todeschini et al., 2018). On day 30 of each trial, the roots and shoots (without berries or flowers) were removed, separated, and stored in a freezer. Then, the plants were dried in an oven at 80 °C for three days. Dry weight (g) of each plant's root and shoot material was measured immediately after removal from the oven.

3.5) Bee Measurements

To consider the viability of *O. lignaria* in different conditions in a vertical farm, we then measured three different indicators of bee health: (1) Bee death rate, (2) Bee reproduction rate, (3) Supplementary food choice test.

Bee death rate acts as an initial indicator of bee stability in the vertical farm conditions. We measured the total number of bees in each room by counting the number of hatched cocoons from the initial 150 set out on Day 1 of each trial. During each day of the trial, we counted and removed the dead bees from Room 2 and 3. With this, we could calculate the cumulative percentage of total dead bees over time. To investigate if death rate differed by the sex of the bee, the dead bees were dated and stored in the freezer; the sex of the dead bees was determined twice a week using past literature on male and female *O. lignaria* differences (Torchio & Tepedino, 1980).

Then, to investigate the reproductive capability of *O. lignaria* in a vertical farm, we also measured the number of completed nests in each room. *O. lignaria* nest in holes and build their nests to consist of mud partitioned compartments which they then complete with a mud cap (Bosch & Kemp 2000). Previous experiments show the preference for the cardboard nesting tubes for holes and overall blue colored nests (Artz et al., 2014). Therefore, in each room, we provided shipping tubes wrapped with blue tape and filled them with approximately 100 cardboard nesting tubes. Autoclaved soil (to prevent introduction of disease into the vertical farm) was provided in a petri dish and moistened every other day. Each day, we observed the tubes and counted the number of capped nests.

Finally, we performed a supplementary food choice test. In addition to the strawberry flowers, bees were provided with four supplementary food choices: (1) 100% Honey bee Pollen, (2) 100% Cattail Pollen, (3) 75% Artificial / 25% Honey bee Pollen, and (4) 25% sugar water (Artz & Pitts-Singer, 2015). Approximately one teaspoon of pollen was added to petri dishes and changed twice a week. Sugar water was provided through a similarly sized liquid bee feeder. We placed the supplements on top of the bucket covers in random order in each room. To examine the most suitable food supplement for *O. lignaria* in a vertical farm, number of visits were counted for 1 minute of each hour the lights were on during the first 15 days of each trial using videos recorded by the Raspberry pi cameras (Artz & Pitts-Singer, 2015).

3.6) Data Analysis

All statistical analysis was completed in R Studio and the package "ggplot" was used to create all figures. To consider if the number of berries produced per plant differed between treatments and season conditions, we used a Zero-inflated regression using the "zeroinfl" function from the "pscl" package (Zeileis et al., 2008). For comparing both the volume and the dry matter content of strawberries between different treatments, a t-test assuming unequal variance was used. A Kruskal-Wallis test was used to check for extreme differences in p-values if the data did not follow a normal distribution (Hollander & Wolfe, 1973). Similarly, we confirmed p-value differences with a one-way ANOVA and Benjamin-Hochberg post-hoc analysis between each treatment. The same tests were used to compare the root:shoot ratio of plants between the spring and summer trials and between room treatments.

To analyze the mortality rate of *O. lignaria* in relation to the Season conditions they faced, we counted the number of hatched cocoons and dead bees and removed them each day. With the number of open cocoons (which usually finished hatching around day 5), the total number of bees in the room was estimated. With this, the cumulative percentage of bees in a room that had died over time was plotted. Once cumulative percentage of deceased bees was plotted over time, two second order polynomial models were fit to the data. One model considered Season grouping and one did not consider grouping by season. These models were compared using AIC scores to determine which was a better fit (Akaike, 1974). Then, the coefficients and associated p-values of the model of best fit were considered.

The same method was used to compare the death rate of *O. lignaria* between the +UV and No UV treatment. Similarly, the death rate of *O. lignaria* was also analyzed between bee sex to understand which sex should be prioritized when protocols are created.

Finally, we analyzed the food supplement choice test observed with the Raspberry Pi cameras. To consider if *O. lignaria* showed a preference for one of the food supplements over the others, a chi-squared goodness of fit test was performed (Lehrer et al., 1995). We then utilized a post-hoc, one-tailed z-test to further understand which of the supplements had a significantly greater proportion of visits. Next, we considered if the proportion of visits to different food supplements was affected by the season conditions the choices were made in. In order to analyze the association between season and supplement choice a chi-squared test for independence was used. If the association was found to be significant, we used the package "corrplot" to create correlation matrix visualizations to identify where the strongest associations were (Wei & Simko, 2017). We repeated the same method to analyze the effect of UV treatment on supplement choice as well.

During all initial analyses, we assumed that starting health of the plants and bees in both spring and summer trials were similar. However, specimens in the summer trial endured a four week longer waiting period which likely affected their health. This complication is discussed in further detail in sections below.

4) Results

4.1) Strawberry and Plant Results

In total, 213 *Fragaria* × *ananassa* plants were grown and observed during the two trials. Although we intended to have 120 plants in both spring and summer trials, some death occurred as plants waited for the summer trial. As a result, only 93 plants were observed in the summer trial (32 in Control, 29 in +Bee, 32 in +Bee/+UV). Over the course of the entire experiment, we harvested a total of 449 strawberries. There were 446 berries in the spring trial and only 3 in the summer trial. Table 1 illustrates the number of berries produced in each treatment during each trial.

As a first attempt to compare the effects of *O. lignaria* pollination on strawberry production, we considered the number of strawberries produced per plant. Figure 1 illustrates the number of berries produced per plant in each of the treatments during each season. Over all three treatments, plants grown during the spring trial produced an average of 3.725 strawberries per plant, and plants grown during the summer trial averaged .032 berries per plant. The number of berries per plant was significantly higher in the spring conditions compared to the summer conditions ($t = 12.76$, $df = 120.64$, p-value $< 2.2e-16$; Zero inflated p-value $= 2.5e-08$). We then compared the number of berries produced between different treatments. Plants grown in the Control room produced an average of 0.75 berries per plant. Plants grown in the +Bee treatment averaged 2.80 strawberries per plant. A significantly greater number of berries was produced in the +Bee treatment compared to the control ($t = 4.62$, $df = 105.91$, p-value $= 1.098e-05$; Zero inflated p-value $= .0008$). Similarly, plants in the +Bee/+UV produced an average of 2.85 berries per plant, a significantly larger amount compared to the Control ($t = 4.71$, $df = 108.41$, p-value $=$ $7.511e-06$; Zero inflated p-value $= .0006$). However, the difference between the number of berries per plant between +Bee and +Bee/+UV treatments was not significant ($t = 0.087$, $df = 137.99$, p-value = 0.93; Zero inflated p-value = .401). These initial results indicate the ability of *O. lignaria* to pollinate strawberry plants with or without UV spectrum lighting.

To further explore the ability of *O. lignaria* to pollinate strawberries in a vertical farm, we compared the volume of strawberries grown in the different treatments. Because only 3 berries were produced in the summer trial, we were only able to compare berry volume between rooms in the spring trial. The spring trial's Control treatment's average berry volume was 312.15 mm^3, +Bee treatment's average was 627.49 mm^3, and +Bee/+UV treatment's average was 685.33 mm^3. Figure 2 illustrates the spread in the volume of strawberries grown in each treatment. Berries grown in the +Bee and +Bee/+UV treatments were significantly larger in volume than those grown in the Control treatment ($t = 4.7293$, $df = 181.88$, p-value = 4.504e-06; ANOVA p-value = 0.008; $t = 5.3668$, $df = 199.84$, p-value = 2.21e-07; ANOVA p-value = 0.003). However, no significant difference was noted in berry volume between +Bee and +Bee/+UV treatments ($t = -0.77999$, $df = 394.43$, p-value = 0.4359; ANOVA p-value = 0.4156). Together, our results suggest that *O. lignaria* presence increases the number of berries and strawberry volume compared to the control, but that *O. lignaria* pollination ability does not improve with UV lighting.

We then compared the dry matter content (DMC) in the strawberries between treatments. Of the 19 plants in the spring trial's Control treatment that produced berries, the average DMC of the strawberries was 15.7%. This was similar to the average DMC in the strawberries from the +Bee treatment at 15.6%, and higher than that of the +Bee/+UV treatment at 12.4%. Figure 3 displays the spread of each treatments' DMC. However, a statistical analysis found that only the DMC between the strawberries of the +Bee and +Bee/+UV treatments differed significantly (t =

2.8103, df = 52.661, p-value = 0.006934; ANOVA p-value = 0.035). The DMC was not significantly different between the Control and +Bee treatment and between the Control and +Bee/+UV treatments (t = -0.04395, df = 32.313, p-value = 0.9652; ANOVA p-value = 0.96; t = -1.8935, df = 20.831, p-value = 0.07226; ANOVA p-value = 0.053).

In order to holistically consider the strawberry plants' health, we compared the root:shoot ratio of the *Fragaria* × *ananassa* plants between both season trials and room treatments. As Figure 4 indicates, the average root:shoot ratio in the summer trial of 1.04 was higher than the average ratio in the spring trial of .413 (t = -0.86864, df = 92.525, p-value = 0.3873, ANOVA p-value =1.1e-10). The higher root:shoot ratio for the summer trial was due to a lower shoot weight in the summer compared to the spring (t = 6.4616, df = 200.85, p-value = 7.676e-10), not a higher root weight (t = -0.75354, df = 177.77, p-value = 0.4521). In addition, a significantly larger spread in the root:shoot ratios was observed between plants in the summer trial compared to the spring (Levene test p-value = 6.372e-07). However, when comparing the root:shoot ratios of plants between treatments, the root:shoot ratio did not significantly differ between Control, +Bee, and +Bee/+UV treated rooms (p-values < .09). There was also no significant difference in the variances of root:shoot ratio between the treatment rooms (p-value < .17). These results indicate a difference in the resource uptake and allocation of the plants depending on the Season conditions, with the presence of O. lignaria having no effect.

4.2) Bee Viability Results

To consider if the spring and summer trials had different bee mortality rates, the cumulative percentage of deceased bees was plotted over time. Figure 5 displays these results along with two second order polynomial regression models fit to the data. Model 1 does not consider Season condition in its fit, while Model 2 does take Season into account, producing one line for spring and one line for summer trials. AIC scores suggest that Model 2 is a better fit (M1 AIC = 454, M2 AIC = 427). Furthermore, the estimated coefficients for Season were significant (p-value < .001). In Model 2, the summer trial line falls above the spring trial line indicating that the bee mortality rate was higher in summer than in spring. These results must be considered conservatively with the longer waiting period the summer trial bees endured kept in mind. This issue is further discussed in the section below.

Similarly, we compared the mortality rate of bees between the presence and absence of UV light. We use Model 1, which has no grouping again. In this case, Model 1 does not consider UV presence in its fit, but Model 3 does take the presence of UV into account, producing one line for +Bee/No UV rooms and one for +Bee/+UV rooms. Figure 6 displays these two models. AIC scores indicate that grouping by UV treatment (Model 3) creates a better fit. (M1 AIC = 454 > M3 AIC = 422). In Model 3, bees without the presence of UV light display a higher mortality rate than those with UV presence. The estimated coefficients for Model 3 are also significant (p-values <0.001). The coefficient for the death rate Model 3 produces for +UV treatment was lower (39.7) than the coefficient Model 3 produces for UV absence treatment (56.2); the +UV death rate is below the no-UV line. This indicates that bees in the presence of UV have a lower mortality rate than those without.

When we similarly consider the difference of mortality rate between male and female bees, male *O. lignaria* had a significantly higher rate of death. The model without grouping for gender produced an AIC score of 338.2; the Model that grouped for gender placed the death rate of males above females and produced an AIC score of 301.3. This result reaffirms past research that indicated the short lifespan of male *O. lignaria* compared to the females (Bosch & Kemp, 2001). This suggests that our focus for protocols should prioritize the health of female individuals as they likely complete most of the pollination.

To understand the reproductive capability of *O. lignaria* in a vertical farm setting, we counted the number of capped nests each day. In both the spring and summer trials, no complete, capped nests were found. However, when checking on the room each day, we observed individuals inside the nesting tubes at almost all times. Photographs of these observations are available in Supplementary Images. During the summer trial, in the +UV treatment two nests with what looked like partially built caps with some mud on them were observed, but they were never completed or capped. The tubes were empty of any cells when thoroughly investigated after the trial ended. Overall, no reproductive capability was observed inside the vertical farm.

We provided four alternative food choices to investigate the best supplement for *O. lignaria* in a vertical farm. In total, of the 1,964 visits counted to the four different food supplements, 71% of the visits were to the ¼ sugar water solution as Figure 7 indicates. This preference for ¼ concentration sugar water was significantly higher than the proportion of visits to all of three of the pollen supplement choices combined (χ^2= 2230.7, df = 3, p-value < 2.2e-16; one tailed z-test p-value < 2.2e-16). After sugar water, honey bee pollen was the second most visited, artificial pollen mix the third, and cattail the least visited (comparing each pollen supplement proportion to each other using a one tailed z-test yielded p-values <.01 for all comparisons).

To consider if season conditions affected the food supplement preferences of the bees, we evaluated if there was an association between trial and supplement visit proportions. As Figure 8 indicates, sugar water was still overwhelmingly the most visited in each season condition. However, a chi-squared test of independence indicates that there is an association between season and supplement visit proportions ($\chi^2 = 46.419$, df = 3, p-value = 4.618e-10). A correlation matrix visualization suggests this is because of differences in visit proportions to honey bee pollen. The visualization indicates a strong positive association of honey bee pollen visits in summer conditions and a negative association of honey bee pollen in spring conditions (Figure 9). Similarly, we also considered if UV treatment affects supplement preference. Figure 10 displays the proportion of supplement visits by UV treatment. The association between UV treatment and supplement visit proportions was also found to be significant ($\chi^2 = 139.62$, df = 3, p-value < 2.2e-16). The correlation matrix visualization of this chi-squared analysis indicates a strong positive association of honey bee visits with +UV treatment and a strong negative association of honey bee pollen visits with No-UV treatment (Figure 11). In contrast, artificial pollen visits were negatively associated with +UV treatment and positively associated with No-UV treatment.

4.3) Room Measurement Results

During the spring trial, temperature was set between 18°-22° Celsius. Room temperature was set between 22°-27° Celsius in the summer trial. However, due to environmental chamber malfunctions, temperatures in the +Bee/+UV room experienced spikes higher than the set ranges for approximately five minutes a day during the transition from night to day temperatures. Supplementary Figures 2 and 3 display these spikes and summarize the temperatures in each of the treatment rooms during both trials. In addition, the average light intensity of each bucket measured from three different points every other day is summarized in Supplementary Figures 4 and 5. Finally, the daily pH measurements taken before any stabilizing additions were added are visualized in Supplementary Figures 6 and 7.

5) Discussion

5.1) O. lignaria Strawberry Pollination

With a global decrease in honey bee health, scientists turn to the pollination ability of different bee species (Bromenshenk et al., 2010; Pettis & Delaplane, 2010). Our results show that strawberry plants grown in the presence of *O. lignaria* produced a higher number of berries than those without (Figure 1). In addition, *O. lignaria* treated rooms had significantly larger berry volumes than those in the control (Figure 2). Greater amount of fruit set and larger fruit size are clear indications of pollination (Wietzke et al. 2018). Taken together with our observations of bees visiting flowers, these findings indicate that *O. lignaria* are, indeed, able to pollinate strawberry plants. Photographs of observed pollination events are available in Supplementary Images. This confirms the single, previous open field experiment that established *O. lignaria*'s strawberry pollination ability (Horth & Campbell, 2018). Moreover, our project indicates that not only are *O. lignaria* able to pollinate strawberries, but also, they are able to do so in a completely indoor, hydroponic farm system. We now move to consider what our results suggest as the best procedures to utilize *O. lignaria* in a VF and the holistic implications of this project on sustainable agriculture.

5.2) UV Recommendations

We did not observe a significant difference in the number of strawberries produced per plant (Figure 1) or the volume of strawberries (Figure 2) between the +Bee and +Bee/+UV treatments. These results suggest that the pollination ability of *O. lignaria* does not depend on UV spectrum light between the range of 380-420 nm that was included in our +UV treatment. Furthermore, *O. lignaria* successfully pollinated strawberries with no light falling below 420 nm.

However, despite the fact we found no differences in the pollination abilities of *O. lignaria* based on UV presence, we discovered that in both seasons, bee mortality rate was lower when in the presence of the 380-420 nm UV light (Figure 6). We predict that UV light, although not necessary for pollination, is required for other behaviors that increase longevity. Other behaviors might include orientation or setting circadian rhythm (Spaethe, 2005). Although little research has been completed on *O. lignaria*'s sensitivity to UV light, a past study indicates the parameters of spectral sensitivity for different bee species in the same family, Megachilidae. *Osmia rufia* has a spectral sensitivity between 344-352 nm and *Chelostoma florisomme* has one between 324-356 nm (Peitsch et al, 1992). Both parameters are lower than the range of our experiment's UV light. Further research is needed on *O. lignaria*'s use of UV light, specifically at lower spectrums. However, even in the +UV treatment, by day thirty, approximately 75% of bees had died. *O. lignaria* have a life span of approximately 5-6 weeks in the wild (Bosch & Kemp, 2001). The benefits of extending *O. lignaria* life spans in a VF to their naturally short life spans with UV addition must be considered with the financial cost of UV and its effects on the crops grown in the farm.

For the strawberries grown with *O. lignaria* pollination, our results indicated a lower dry matter content (DMC) in berries grown in UV spectrum light compared to those without UV (Figure 3). DMC is a good estimator of the sugar and starch content in a fruit, so these results suggest that strawberries grown in UV spectrum light are lower in these regards. Studies completed on other crops, such as corn, indicate increased UV light can cause a reduction in a crop's chlorophyll content (Gao et al. 2004). This causes a reduction in plant photosynthetic efficiency. We suspect that the strawberry plants under UV light similarly had reduced photosynthetic ability, so produced less sugars and starches in their berries. More light overall can also lead to higher transpiration and an increased water uptake in the berries. DMC is an important factor of fruit taste. Past research on apples illustrates a positive correlation of DMC and consumer preference; a lower DMC lowers fruit taste (Palmer et al. 2010). However, the control treatment (No Bee, No UV) had an average DMC of 15.7%. This was not found to be significantly higher than the +Bee/+UV treatment's average DMC of 12.4%. But, with only 19 plants producing berries in the control, the sample size was low. This discrepancy indicates a need for future studies to more clearly establish the effects of UV light on strawberry DMC and the overall needs for UV spectrum when growing different crops indoors.

Our results suggest a tradeoff between *O. lignaria* longevity and strawberry DMC with the presence of UV light. However, with such a short lifespan regardless of UV light, *O. lignaria*'s ability to pollinate without UV, and lower strawberry DMC in the presence of UV, we recommend careful to no use of UV spectrum when working with *O. lignaria* and strawberries in a VF. Moreover, with high energy costs already posing both a financial and environmental issue for VFs, the recommendation to not use UV also assists in lowering that strain (Yu et al., 2013). Unfortunately, our experiment did not have the resources to include a No Bee and +UV treatment. We encourage further investigation of UV spectrum light in relation to both *O. lignaria* and VF grown strawberries to build upon these preliminary results.

5.3) Season Condition Recommendations

Between the two season trials, we observed a large difference in the number of berries produced per plant. The spring trial produced a significantly higher number of berries per plant compared to the summer regardless of bee or UV presence (Figure 1). We expected similar fruit amounts in both seasons from this Day Neutral, Cabrillo strawberry, as this cultivar is advertised as being able to produce fruit if the temperature remains from 1° to 30°C (Boeckmann, n.d.).

Additionally, the UC Davis site (who patents this cultivar) suggests the Cabrillo cultivar is adapted to standard fall planting in addition to spring and summer planting and should produce every season ("The UC Patented Strawberry Cultivars," n.d.). However, our results indicate that spring-like conditions (18 °C - 22 °C, 14 hours photoperiod) were the most suitable for this plant in a vertical farm. We must also consider that the strawberry plants in the summer trial endured four weeks longer of waiting than those in the spring trial; this issue is discussed further below.

Comparing the mortality rate of *O. lignaria* in different season conditions demonstrated that *O. lignaria* death rate was higher in the summer trial than the spring (Figure 5). These findings suggest that a vertical farm temperature between 18 °C to 22 °C and shorter light period is more suitable for *O. lignaria* health than a higher temperature between 22°C to 27 °C and longer light period. In the wild, *O. lignaria* begin emerging from their cocoons in early April and are active between mid-April to mid-June. They have been observed foraging and pollinating in temperatures as low as 12 °C (Bosch & Kemp, 2001). *O. lignaria* may not be as effective pollinators in warmer temperatures if adapted to emerge in Spring. Moreover, *O. lignaria* have been shown to use temperature as a cue to induce behaviors when inside cocoons, so *O. lignaria* may be receiving confusing environment cues when emerging from cocoons in the unexpectedly high temperatures. Too few berries were produced in any of the treatments of the summer trial to adequately see *O. lignaria's* ability to pollinate in summer heat, but from their mortality rate, we suggest the use of *O. lignaria* to be limited to crops needing cooler, spring-like temperature.

The plants grown in the summer trial had a higher average root:shoot ratio than the plants grown in the cooler spring trial (Figure 4). Land plants utilize shoots to capture solar energy and atmospheric carbon for photosynthesis and depend on roots for the acquisition of water and nutrients (Wang & Ruan, 2015). The higher root concentration compared to shoot concentration in the summer trial indicates the plants' needs for either more water uptake, more mineral uptake, or both. (Sharma et al., 2018). At higher temperatures water evaporates more rapidly, and therefore plants transpire more rapidly, which would lead to the need for more water if temperature was too high. However, we observed the higher root:shoot ratio in the summer trial to be caused by a lower shoot weight, not a higher root weight. Past research on Sonata strawberries has found an increased allocation of dry matter into leaves and less into roots at higher temperatures. In this study, plants showed an increased shoot content at 24 °C than at 18 °C (Sønsteby et al., 2016). Our conflicting result suggests an overall lower quality of health than expected in the summer trial. The higher variance in the ratio we observed in the summer trial supports the suspicion of variability in the health of this trial's plants.

Both the plants and bees measured in the summer trial endured a four week longer waiting period than those used in spring. This longer waiting period likely caused a decrease in health for both subjects in the summer trial (Bosch & Kemp, 2003). Also, one bucket in the +Bee/+UV treatment in the summer trial experienced minor root rot due to a broken air pump; the water and nutrients in this bucket were completely changed during Week 2. Our comparisons between the spring and summer trials do encourage the use of spring conditions for the use of *O. lignaria* pollination in a strawberry VF. However, our results and recommendations must be taken conservatively given the issues mentioned in the summer trial. For vertical farms overall, shorter light hour recommendations mean less operating energy costs, but maintaining the spring temperature may require either more or less energy (more cooling or less heating) depending on the climate the VF resides in. Vertical farms must also take these issues into account.

5.4) Sex, Reproduction, and Food Supplement Recommendations

Collectively, our analysis indicated that male *O. lignaria* had a higher mortality rate than females in our experiment. On average, by day 20 of the trial, over 80% of male bees had died compared to approximately 50% of the female bees. We expected this to be the case; although males outnumber females about 2 to 1, males emerge one to three days earlier than females and die soon after mating with newly hatched females who emerge a few days later (Bosch & Kemp, 2001). We observed this to be the case in a vertical farm setting as well. Males hatched first, and then we observed multiple mating events in the following days when females began emerging. Past literature notes that male *O. lignaria* only visit flowers to collect nectar and pollen for their own consumption, whereas females visit many flowers for the construction and provisioning of nests (Bosch & Kemp, 2000; Bosch & Kemp, 2001). Our mortality rate results in addition to past research on sex differences, suggests that any procedures for *O. lignaria* pollination should focus on the health of female *O. lignaria* as they will complete most of the pollination after mating with short lived males.

During the entire experiment, we did not observe any completed nests in any of the rooms. *O. lignaria* nests are unique because a single nest hole will be partitioned into multiple cells containing an egg and pollen-nectar provision using mud (Bosch & Kemp, 2001; McKinney & Park, 2012). In the outdoors, females with a life span of 20 days will provision two to four nests on average (Bosch & Kemp, 2001). We observed multiple mating events and bees inside the provided nesting cavities at almost all times, but found no completed, capped nests or mud partitions. This suggests the bees identified the nesting tube set up but that some necessary resources or environmental cues were missing. We provided autoclaved soil in each of the rooms and moistened the soil every other day, but rarely observed bees interacting with the dishes of soil. We did identify small amounts of soil in the nests during the summer trial, but we suspect using a different type of soil or keeping the soil moister might be necessary. Soil in Utah, where the bees are sourced from, is very alkaline and clay-like; our soil in the experiment was not. Overall, this experiment did not display reproductive capability in a VF but encourages more research on the subject as the observed mating events, traces of soil, and identification of nests suggests reproduction in a VF is possible with further research.

As we expected, sugar water, the one nectar supplement, was the most visited choice among the four food supplements (Figure 7). Pollen and nectar differ in their nutritional quality. In addition, *O. lignaria* mainly collect pollen only for provisioning brood, but nectar is collected both for personal consumption and nest provisioning (Bosch & Kemp, 2001). Since male *O. lignaria* do not participate in provisioning, they only visit flowers and food sources to collect nectar for their own consumption (Bosch & Kemp, 2001). Honey bee pollen was the most visited of the three pollen supplements, but interestingly, it was positively associated with summer and +UV conditions (Figure 8 & Figure 10). We theorize this association might mean honey bee pollen is more desirable under stronger health strains as seen in the summer, but if this were entirely the case, we would expect positive association with the No UV treatment too. Further development is needed on this subject. Our analysis suggests *O. lignaria* most prefer a nectar supplement (like 25% sugar water) in a VF. While it must be considered that results may have been affected by which pollen supplement was randomly placed next to the sugar water feeder, honey bee pollen was the most favored pollen in this experiment. Because little cost is associated with providing both sugar water and honey bee pollen supplements, we suggest the addition of both when using *O. lignaria* is a VF. However, further research is needed to clearly establish if preferred pollen types vary by environmental conditions or quality of health.

5.5) Experimental Caveats

As mentioned, the largest issue of our project was the longer waiting period the plants and bees investigated in the summer trial endured. We assume all bees began their adult wintering stage around the same time in autumn of 2018, meaning summer trial's *O. lignaria* experienced a much longer wintering duration. Longer duration of wintering can negatively affect the health upon emergence and longevity of *O. lignaria* because they run out of fat stores during long wintering periods. Lowering the storage temperature to 0 °C may alleviate some of this pressure (Bosch & Kemp, 2003). We had hoped *O. lignaria* could be stored in adult wintering phase for long periods for VF use. However, just like year-round use of Sweat Bees requires the continual starting of new colonies, year-round use of *O. lignaria* may require continual release, nest collection, pre-wintering, and wintering of cocoons to ensure bees do not face too long of wintering storage (Greenberg, 1982). Further development is needed on these indoor procedures.

Furthermore, our experiment did not have the resources to include a No Bee/+UV treatment room. The addition of this room would be beneficial to more clearly differentiate between the effects of UV spectrum light and *O. lignaria* on strawberry plant productivity. Due to time constraints, we also had to end the trial before many berries were ripe. This may have caused some differences in the volume and DMC trends we analyzed in this study.

Although each light was set approximately 1 meter away from its bucket, some differences in light intensity were measured between shelves because of different reflection from room walls (Supplementary Figure 4 & 5). This may have caused some unaccounted for differences in plant growth and berry production. Moreover, the +Bee/+UV treated room experienced some unexpected spikes in temperature when changing from the set night to day temperatures

(Supplementary Figure 2 & 3). This spike was brief but may have caused additional strain for the test subjects in the +Bee/+UV room and altered their productivity or health.

Because we used already germinated strawberry plants in this experiment, we noticed some pests in our farm. Small numbers of thrips *(Thysanoptera)* were noticed in each of the rooms during both trials. Small amounts of black gnats (either fruit flies or fungus gnats) were also observed. We suggest growing strawberries from seed in future studies to best avoid outside pests. We also noticed a few incorrect bee species during the experiment due to being sent some misidentified cocoons from the source. These individuals were identified as closely related *Osmia californica* and were removed as quickly as possible.

5.6) Future Direction and Vertical Farms

Strawberries are grown on over 60,000 acres in the United States (Demchak & Harper, 2005). Most of this production occurs in California where crop production places a large strain on natural water systems. Growing strawberries in vertical farms provides a unique opportunity to reduce the environmental strains of this beloved crop while progressing overall sustainable agriculture methods. This project confirms *O. lignaria*'s ability to pollinate strawberries in a VF but indicates potential shortcomings of their VF suitability. Sourcing *O. lignaria* proves difficult after spring. In addition, *O. lignaria* life cycles may not be best suited for the continuous flowering of everbearing strawberries or the year-round harvest of other crops vertical farms want to produce. Our experiment did not observe any reproductive ability, and indoor rearing of closely related *Osmia* species has so far proven difficult with high mortality rates (van der Steen, 1997). We confirmed that storing *O. lignaria* in their wintering phase year-round is not feasible either (Bosch & Kemp, 2003). Vertical farms promise to grow plants year-round using completely controlled environments, but *O. lignaria* may not be suitable to pollinate this promise unless continual indoor rearing methods are quickly established.

Further research is needed not only on *O. lignaria* pollination but also on other bee species or potential pollination techniques that could prove useful for VFs. Other solitary bee species closely related to *O. lignaria* but with easier to control life cycles could provide an avenue for continued research. In addition, bumblebees (*Bombus terrestris*) are often used in temperature controlled greenhouses for the production of crops such as tomatoes, and unlike *O. lignaria*, research exists on the year-round rearing of bumblebee colonies (Vergara & Fonseca-Buendía, 2012; Ahmad et al., 2015; Heemert et al., 2015). However, bumblebee circadian rhythms and foraging is highly affected by UV light which must be considered for vertical farm suitability (Chittka et al., 2013). In addition, social bees, as mentioned previously, may be more aggressive in a small VF. While bumblebees may be an avenue for research, we advocate for more investigation on different solitary species as well. This will need to include research on solitary species natural life cycles and how to continually rear them indoors. On the other hand, one alternative study displays an ultrasonic pressure device that can pollinate strawberries in VFs; technology-reliant pollination techniques could potentially lower labor costs with further development too (Shimizu & Sato, 2018).

This project investigates a single opportunity to reduce costs and increase crop production in vertical farms. However, like all novel agricultural solutions, vertical farms face more than just a single hurdle to becoming the sustainable food system of the future. In addition to high labor costs, vertical farms have incredibly high energy requirements (Yu, Yang, & Shimamura, 2013). The ability to source this energy sustainably is often more difficult than expected (Kalantari et al., 2018). Depending on how much more food a VF can produce on the same amount of land, these energy requirements can actually make VF crops less sustainable than traditional agriculture. In his pioneering book, Despommier optimistically predicts that a vertical farm could multiply the amount of food produced on a plot of land by 16 times per floor of the farm (2009). However, in practice this number is much lower and dependent on the specific crop (Yu, Yang, & Shimamura, 2013). Our study investigates a single, specific method to reduce costs and increase food production. But this is not enough. We indicate the need for further research on this promising agriculture strategy that is still in its nascent stages. But more than anything, we advocate for more focus on the global issues of our food system.

6) Acknowledgements

I am most grateful to Professor Sarah Kocher and Dr. Paul Gauthier for their incredible mentorship and guidance with this research. This has been a remarkable learning experience which I truly appreciate.

Thank you to Eli Wyman, Kennedy Saitoti, and the entire Kocher Laboratory. I would also like to acknowledge the Pringle Laboratory, Firestone Data and Statistical Services, Jesse Saunders, and Stephen Nappa for their assistance at different points in this project. I appreciate Professor Dan Rubenstein, Smitha Haneef, and Shana Weber for cultivating and nourishing my passion for food production during my Princeton career.

Thank you, McKenzie Lowe, Gentry Lowe, Annalou Wren, and Mark Kim for their invaluable encouragement and meaningful revisions. Thank you, Justin Hamilton and Kevin Jeon for their initial actions (not initiations) and constructive edits. Thank you, also, my amazing roommates who for the past four years have given me so much support.

This project was funded by the Fred Fox Class of 1939 Fund, the Ecology and Evolutionary Biology Department, the Office of Undergraduate Research, Princeton's Global Health program, and the Princeton Environmental Institute. I appreciate their support in making this project possible.

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8) Tables

TABLE 1: **Total Number of Strawberries Produced in each Treatment and Season Trial**

Berries were picked either when ripe or at the end of the thirty-day trial regardless of ripeness.

	Number of Berries	
Treatment	Spring Trial	Summer Trial
Control	49	1
+Bee	192	0
+Bee/+UV	205	2
Total	**446**	**3**

Note: 120 Plants in Spring, 93 Plants in Summer due to some plant death

9) Figures

FIGURE 1: **Number of Berries Produced per Plant.**

Berries were picked and counted when ripe or at the end of the thirty-day trial. Overall, an average of 2.11 berries per plant were produced per plant.

Seasons: Spring n= 120, Summer n= 93. Averages are Spring: 3.73, Summer: .03 (berries per plant). A Zero inflated test yield p value below .01 comparing Spring and Summer Trials.

Treatments: Control n= 73, +Bee n= 69, +Bee/+UV n= 71. Averages are Control: .753, +Bee: 2.8, +Bee/+UV: 2.85 (berries per plant). A Zero inflated test yields p values below .05 for Control compared to +Bee and +Bee/+UV, but is not significant for +Bee compared to +Bee/UV

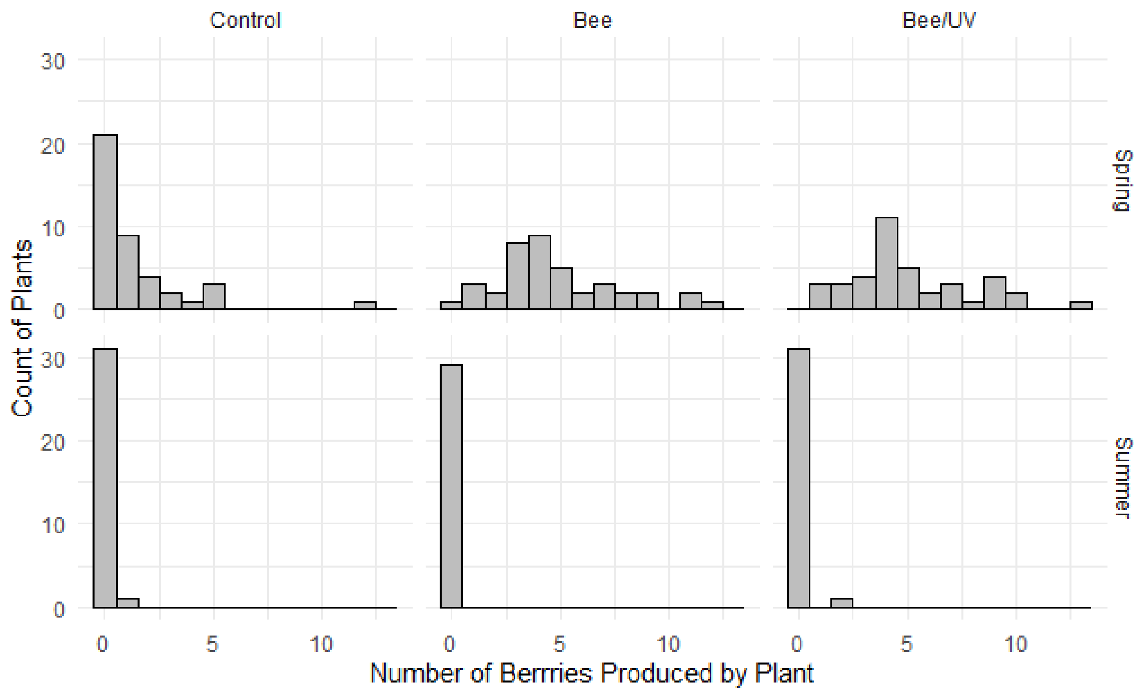


FIGURE 2: **Strawberry Volumes (mm^3) by Treatment in the Spring Trial**

Volume was calculated from the height and width measurements of each berry.

Control n= 49, Bee n= 192, Bee/UV n= 205. Averages are Control: 312.14, +Bee: 627.49, +Bee/+UV: 685.33 (mm^3). A t-test yields p-values of Control & Bee: 4.50e-06*, Control & Bee/UV: 2.21e-07*, Bee & Bee/UV: 0.435.

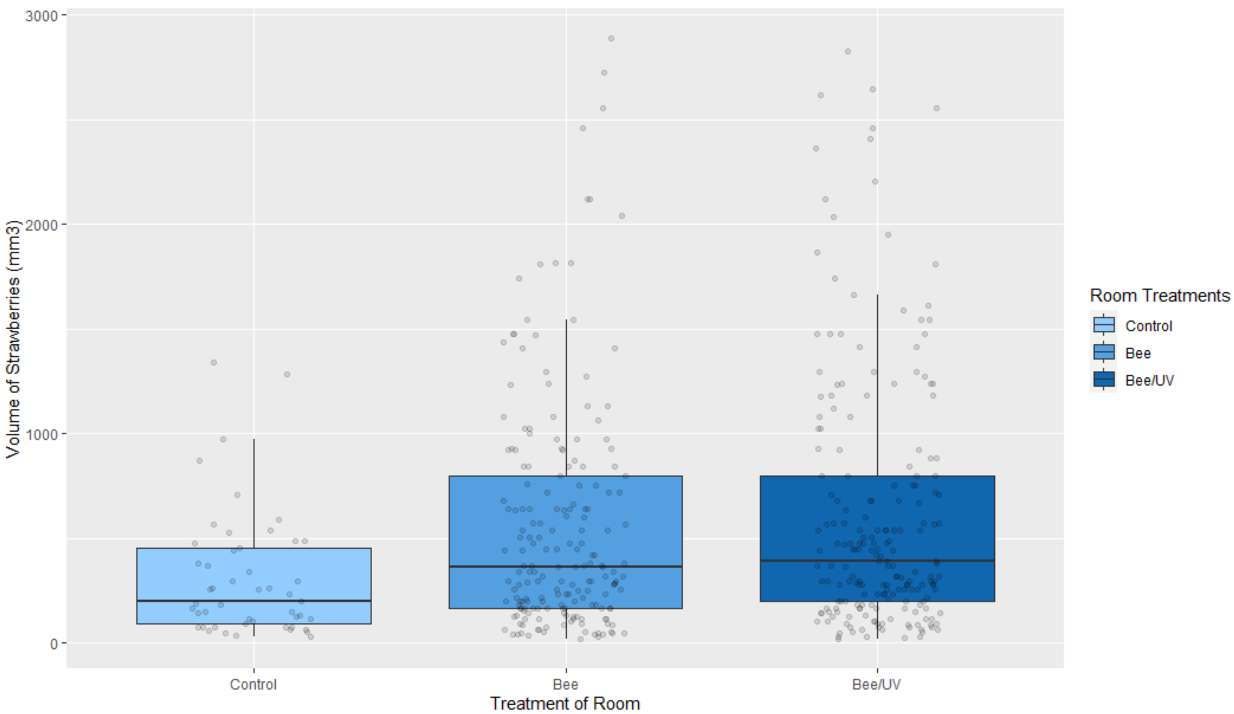


FIGURE 3: **Strawberry Dry Matter Content in the Spring Trial**

Strawberries were weighed immediately after picking and again after 48 hours in a drying oven. Dry Matter Content (% Dry Matter) was pooled for each plants' strawberries.

Control n= 19, Bee n= 39, Bee/UV n= 40. Averages are Control: 15.67%, +Bee: 15.58%, +Bee/+UV: 12.41%. A t-test yields p-values of Control & Bee: 0.965, Control & Bee/UV: 0.053, Bee & Bee/UV: 0.007.

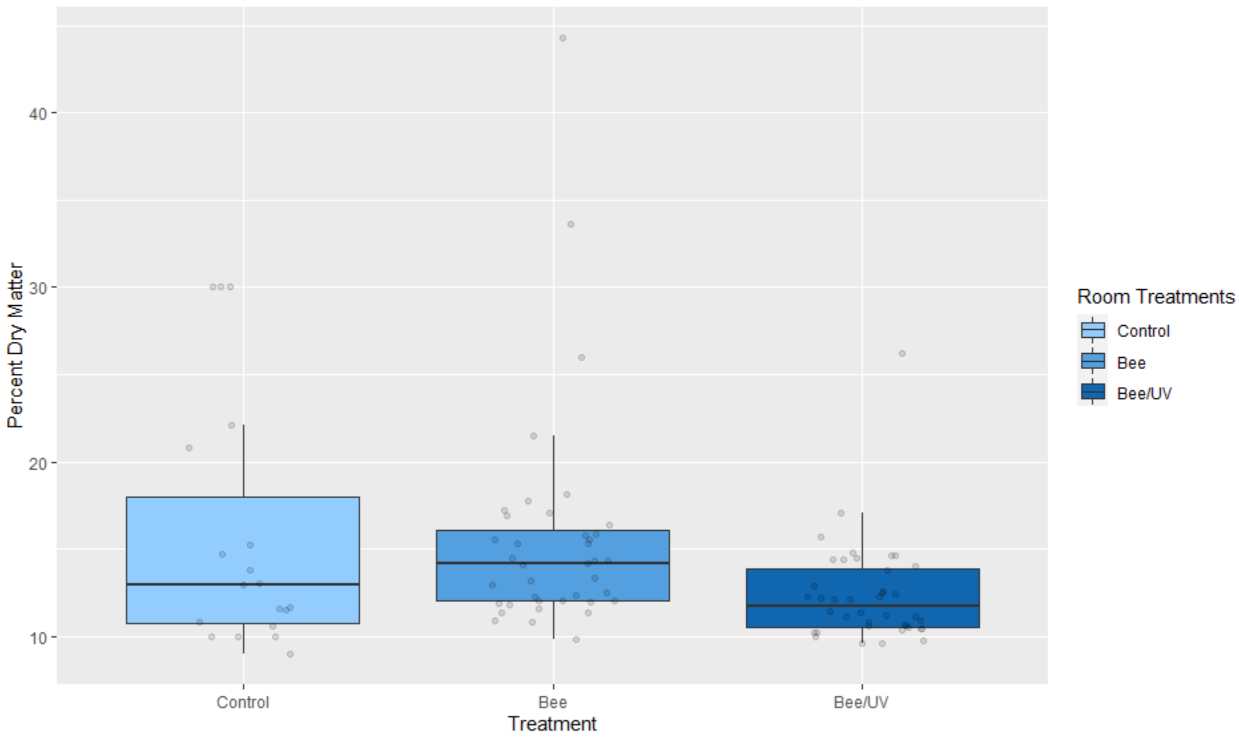


FIGURE 4: **Root:Shoot Ratios of Strawberry Plants by Treatment and Season Trial**

Each plant's root and shoot mass was weighed after 72 hours in a drying oven.

Seasons: Spring n= 120, Summer n= 88. Averages are Spring: .414, Summer: 1.04. A t-test yields p-value = 7.64e-08 comparing Spring and Summer Trials.

Treatments: Control n= 69, +Bee n= 69, +Bee/+UV n= 70. Averages are Control: .675, +Bee: .582, +Bee/+UV: .776. A t-test yields p-values above .09 for all comparisons of treatments.

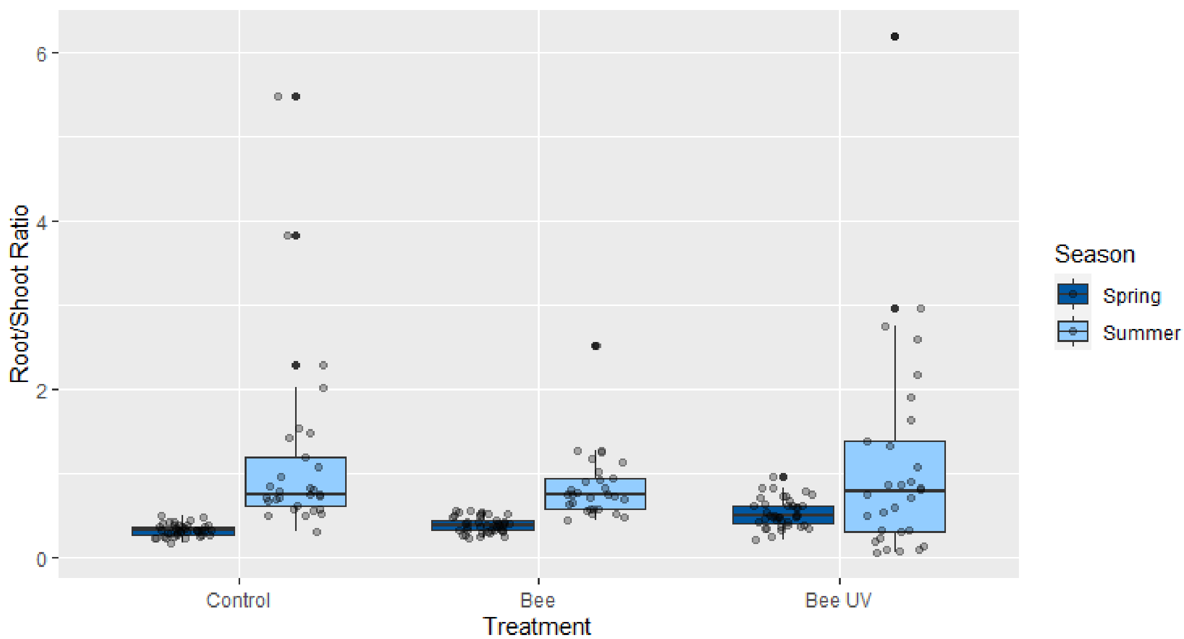


FIGURE 5**: Bee Mortality Rate by Season Trial**

The Cumulative Percentage of Deceased Bees was graphed over Time.

Model 1 (Black) does not consider Season conditions into its fit. Model 2 includes grouping for Season Trials (Light Blue = Summer, Dark Blue = Spring). Model 2 is considered a better fit with a lower AIC score (M1 AIC 454 > M2 AIC 427). Bee mortality rate is higher in the Summer Trial than the Spring Trial.

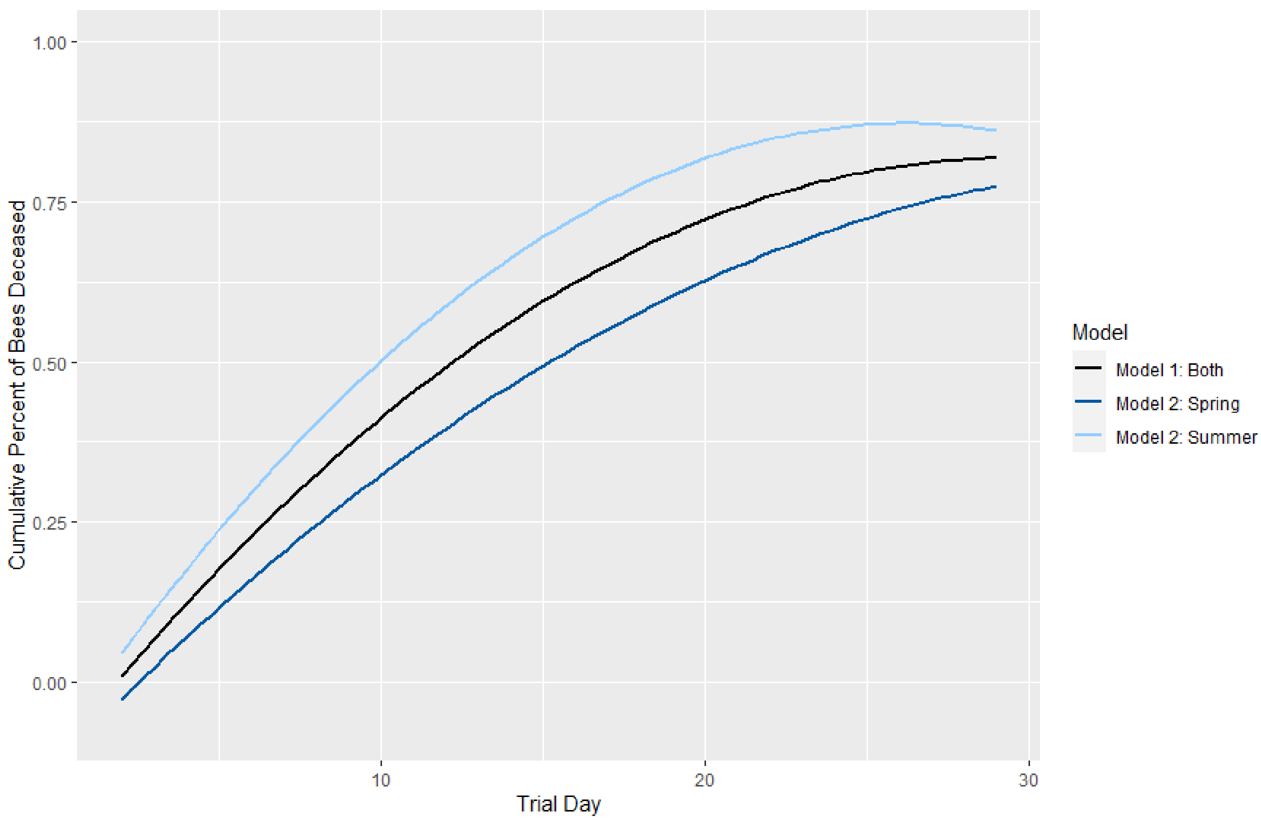


FIGURE 6**: Bee Mortality Rate by UV treatment**

The Cumulative Percentage of Deceased Bees was graphed over Time.

Model 1 (Black) does not include UV light presence or absence into its fit. Model 3 included grouping for UV (Light Blue = No UV, Dark Blue = +UV). Model 3 is considered a better fit (M1 AIC 454 > M3 AIC 422). According to the model, bee mortality rate was lower in the presence of UV spectrum light.

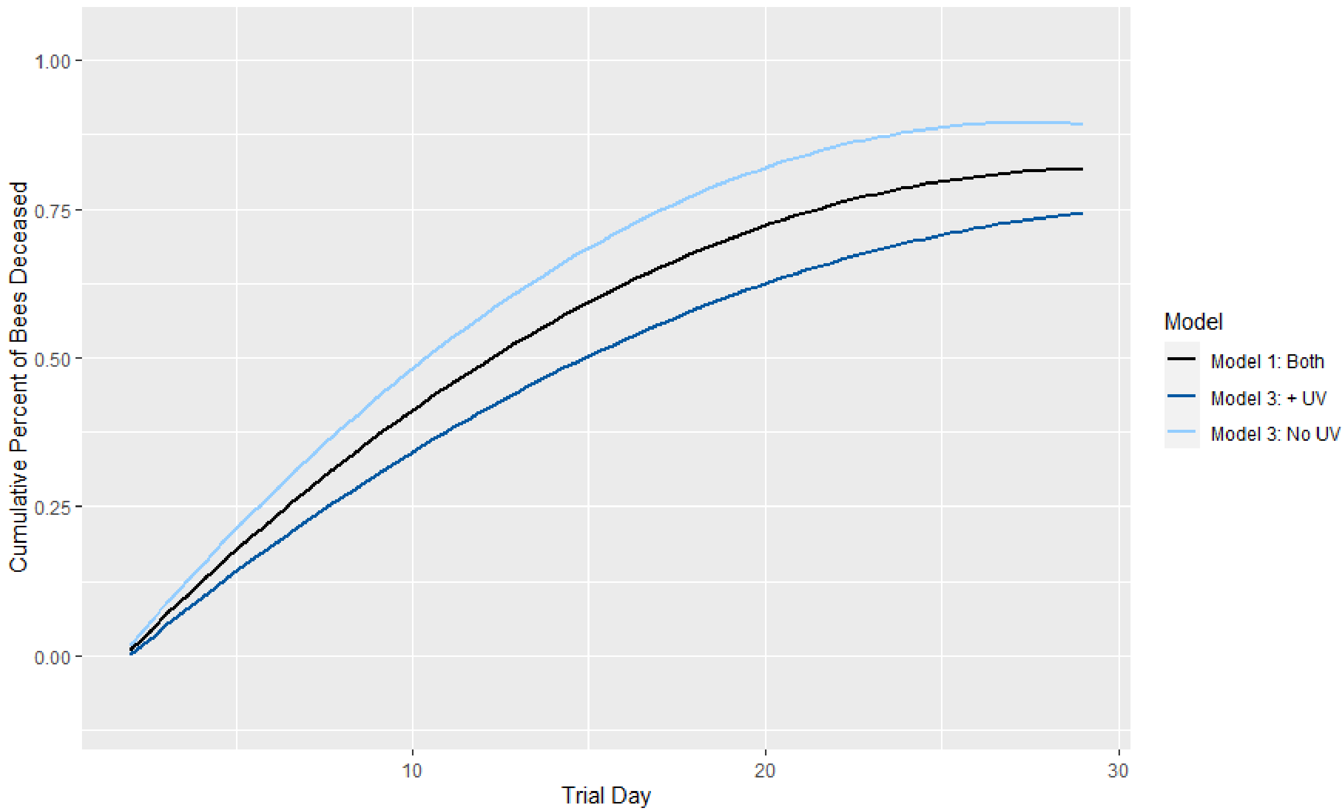


FIGURE 7**: Supplement Visit Proportions**

Visits were counted from 1 minute of video taken every hour lights were on during the first fifteen days of both trials.

N = 1,964. Proportions are Sugar Water: .71, Artificial Pollen Mix: .10, Cattail Pollen: .05, Honey bee Pollen: .14.

A chi-square goodness of fit yields p-value < 2.2e-16. A one-sided z-test comparing proportion of sugar water visits to all other supplement visits yields p-value < 2.2e-16.

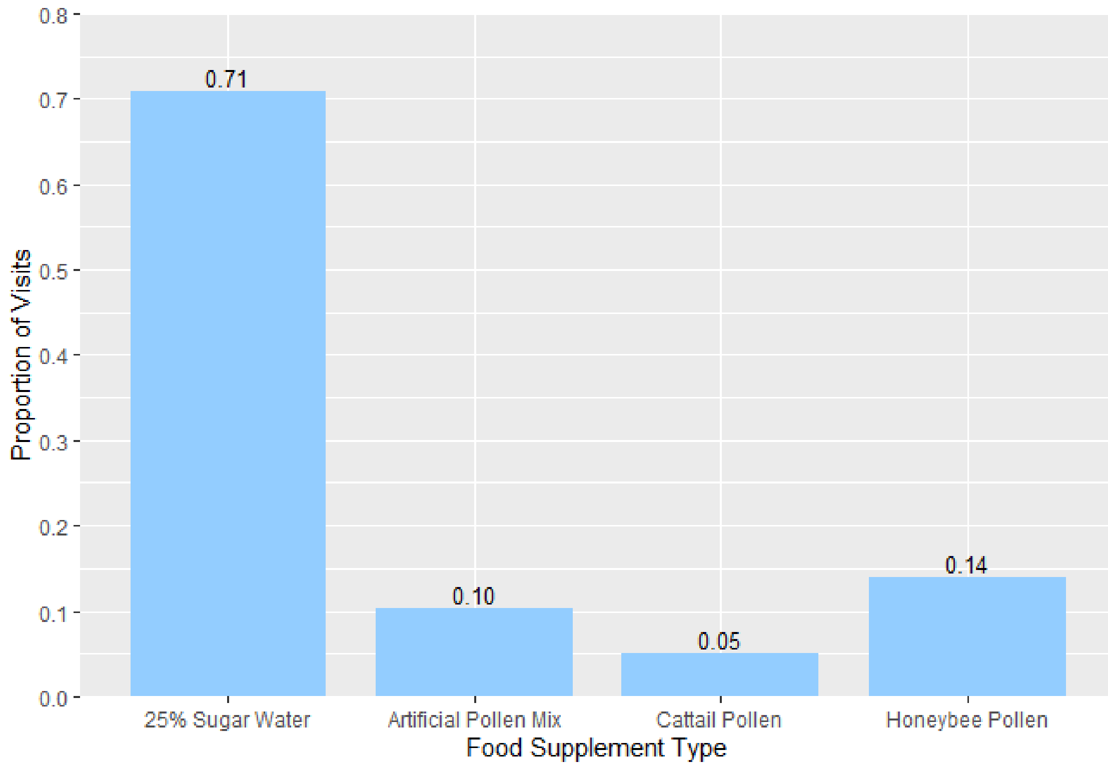


FIGURE 8**: Supplement Visit Proportions by Season**

Spring n=1192, Summer n =772. Proportions are included in the figure.

A chi-squared test of independence indicates there is an association between season and supplement visit proportions (p-value = 4.618e-10)

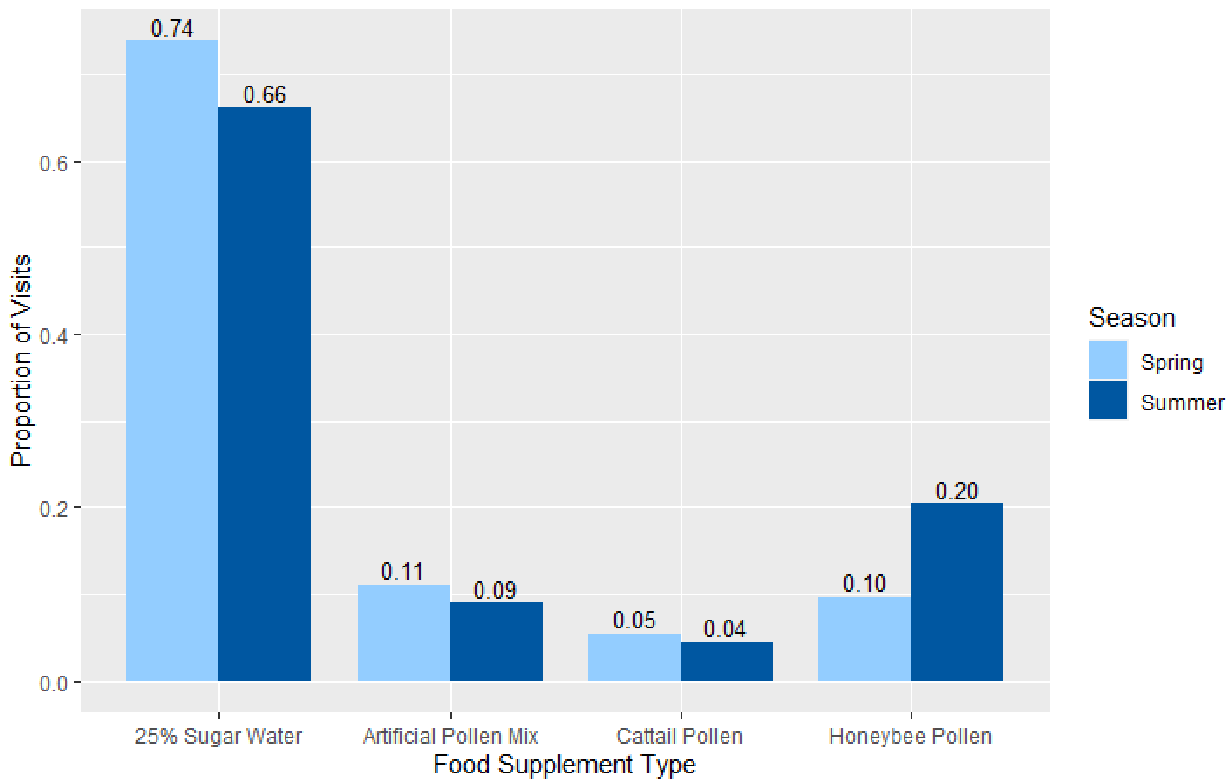


FIGURE 9: **Correlation Visualization of Supplement Visit Proportions by Season**

A correlation visualization created using R package “Corrplot.” A large red circle indicates a negative association, and a large blue circle indicates a positive association. Strongest positive and negative associations were found with season conditions and honey bee pollen proportion.

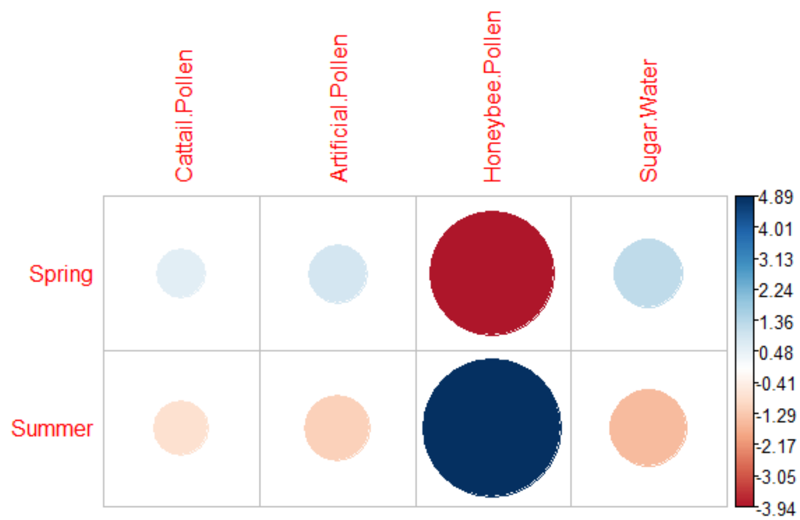


FIGURE 10**: Supplement Visit Proportions by UV Treatment**

+UV n= 1158, No UV n = 806. Proportions are included in the figure.

A chi-squared test of independence indicates there is an association between UV treatment and supplement visit proportions (p-value < 2.2e-16)

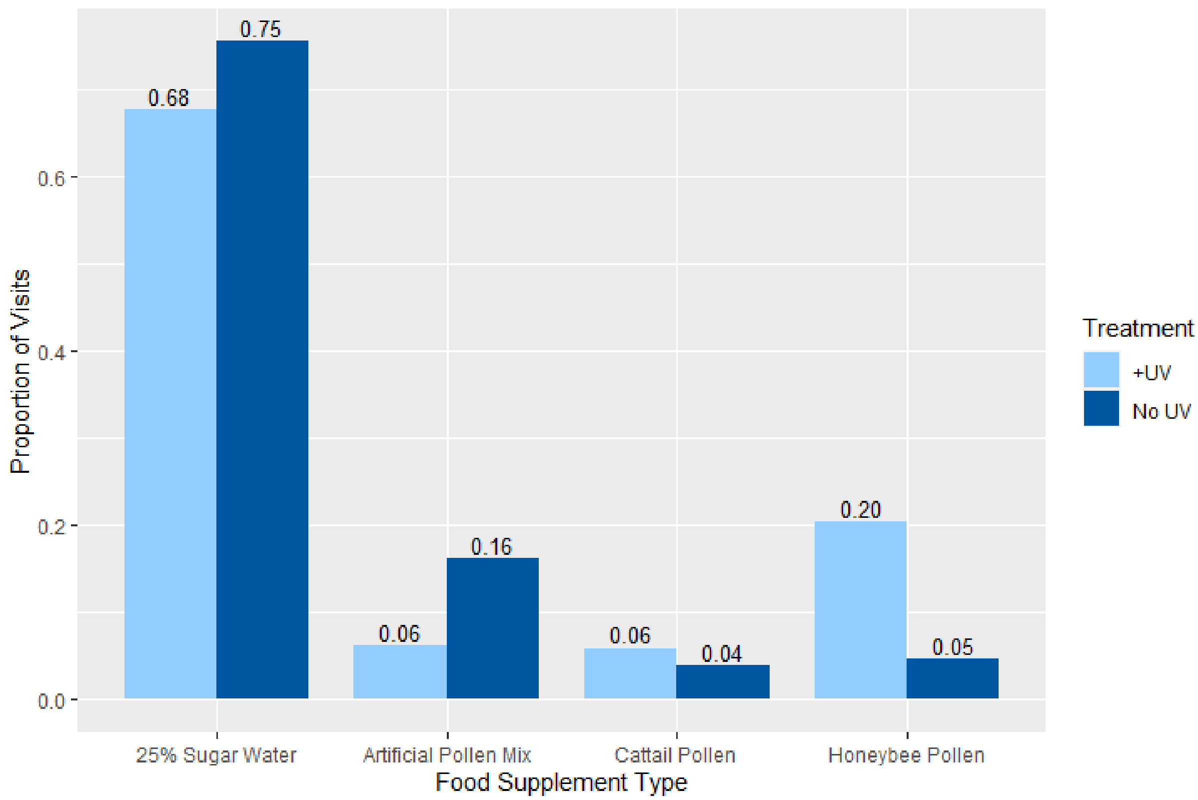
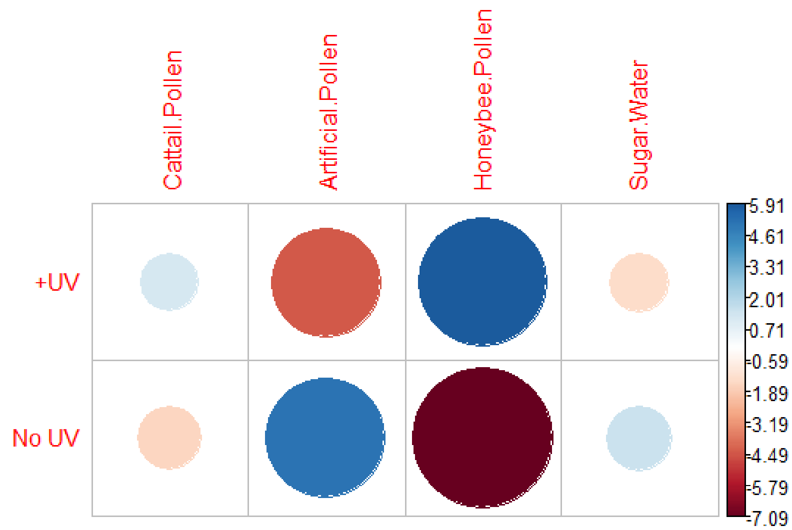


FIGURE 11: **Correlation Visualization of Supplement Visit Proportions UV Treatment**

A correlation visualization created using R package "Corrplot." A large red circle indicates a negative association, and a large blue circle indicates a positive association. Strongest positive and negative associations with UV treatment categories were found with honey bee pollen proportion.



10) Supplementary Materials

SUPPLEMENTARY TABLE 1: **Hoagland Nutrient Solution Composition**

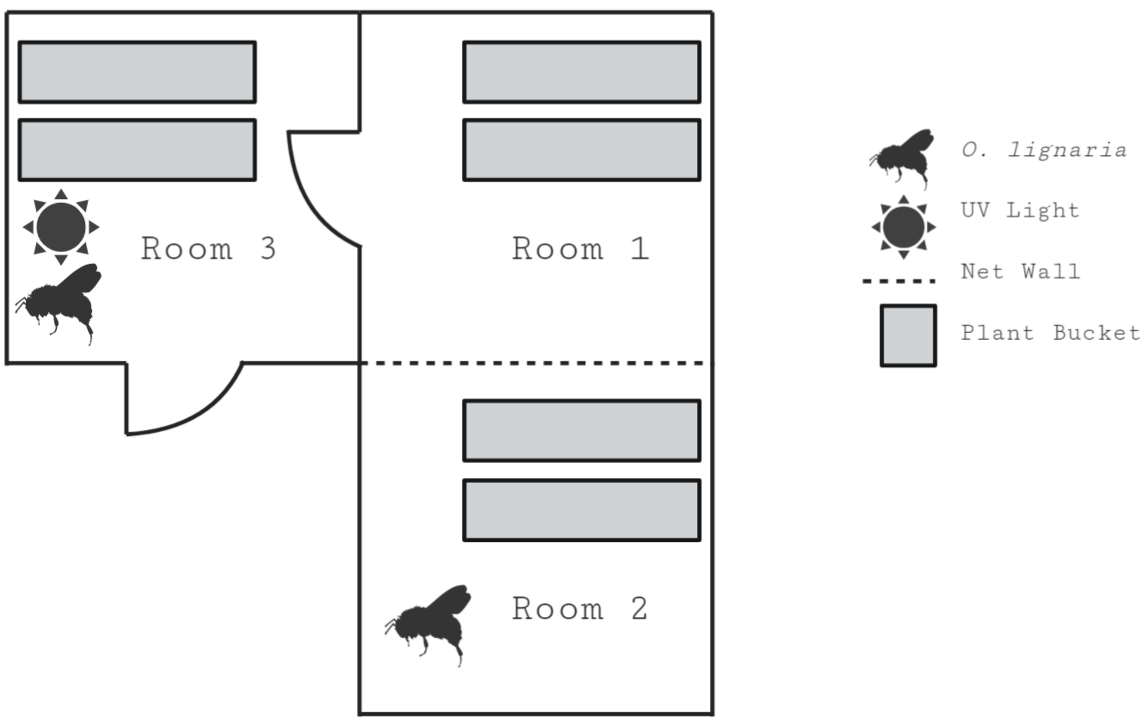
The amount of each nutrient added at the start of each trial to create a 16L solution for each bucket of strawberries. In addition, 150 ml of Calcium Nitrate and 6 ml of Iron were added to each bucket during week three because plants began to look a little yellow. 50 ml of MGSO4 was also added to each bucket during Week 2.

Chemical	Volume Added (ml)
$Ca(NO_3)_2.4H_2O$	32
$(NH_4)H_2PO_4$	32
KNO_3	51
$MgSO_4.7H_2O$	32
H_3BO_3	8
$MnCl_2.H_2O$	8
$ZnSO_4.7H_2O$	8
$CuSO_4.5H_2O$	3
MoO_3	8
$Co(NO_3)_2.6H_2O$	3
NaOH	32
FeEDTA (100x)	80

Note: Values based on personal communication with Paul Gauthier 2019 to be added to 16 L water

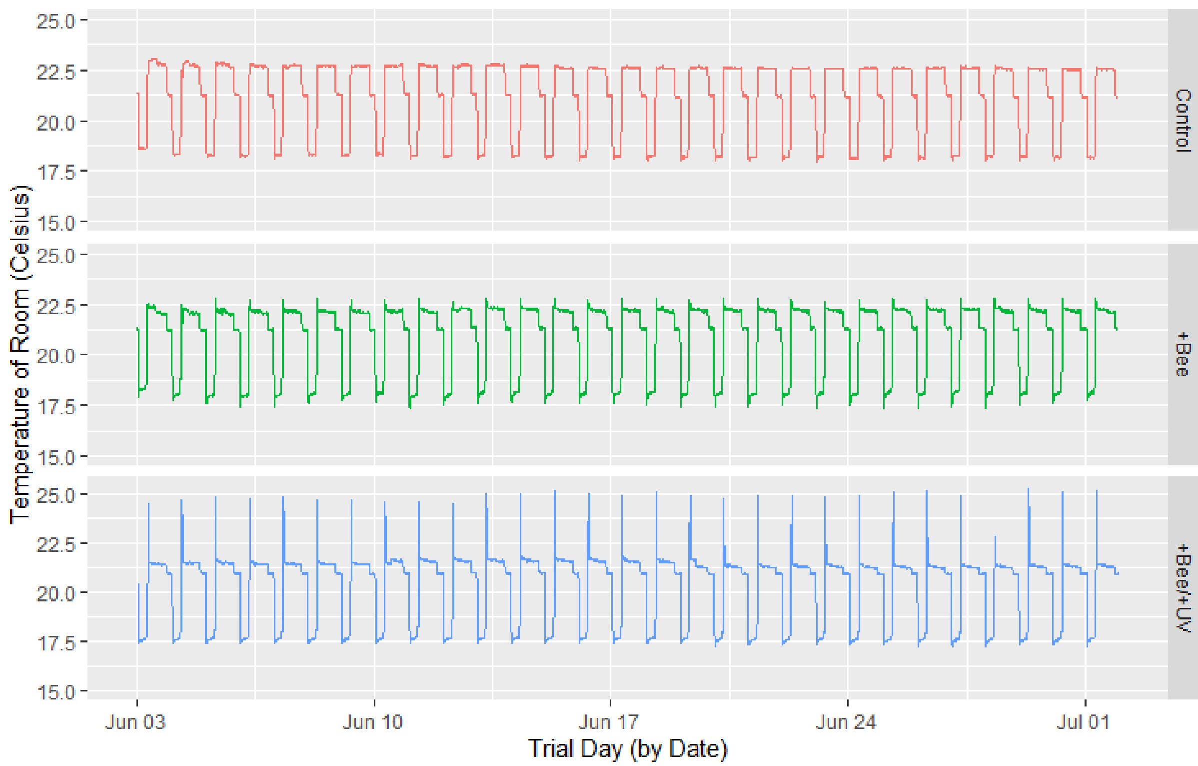
SUPPLEMENTARY FIGURE 1: **Setup of the Three Rooms**

This room treatment set up was kept the same between both trials. Each plant bucket grew approximately 20 strawberry plants.



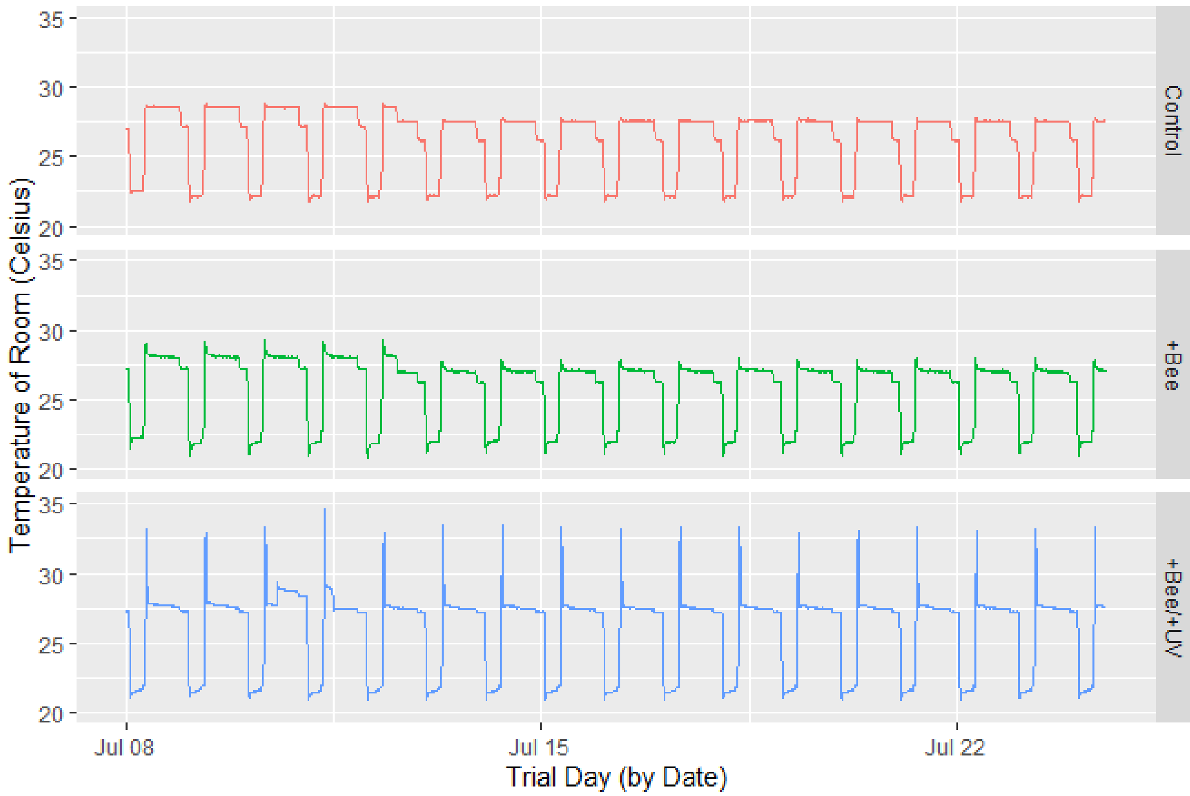
SUPPLEMENTARY FIGURE 2: **Temperature Summary of the Spring Trial**

In each treatment, temperature was recorded by an Apple Homekit Eve Degree® Monitor every ten minutes. The +Bee/+UV environment simulation chamber experienced a short spike (reaching about 24°C) when transitioning from night to day temperatures.



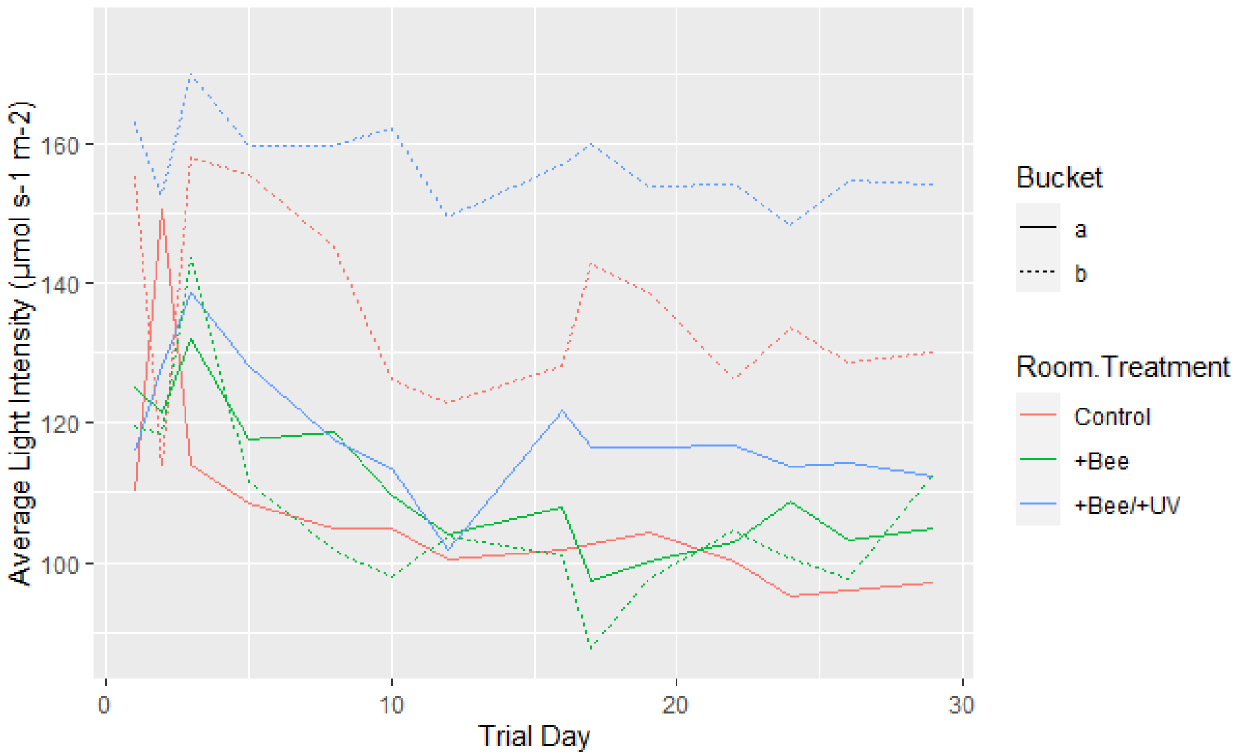
SUPPLEMENTARY FIGURE 3: **Temperature Summary of the Summer Trial**

In each treatment, temperature was recorded by an Apple Homekit Eve Degree® Monitor every ten minutes. Again, the +Bee/+UV environment simulation chamber experienced a short spike (reaching about 33°C) when transitioning from night to day temperatures. Temperature is not included for the final week of this trial but remained the same.



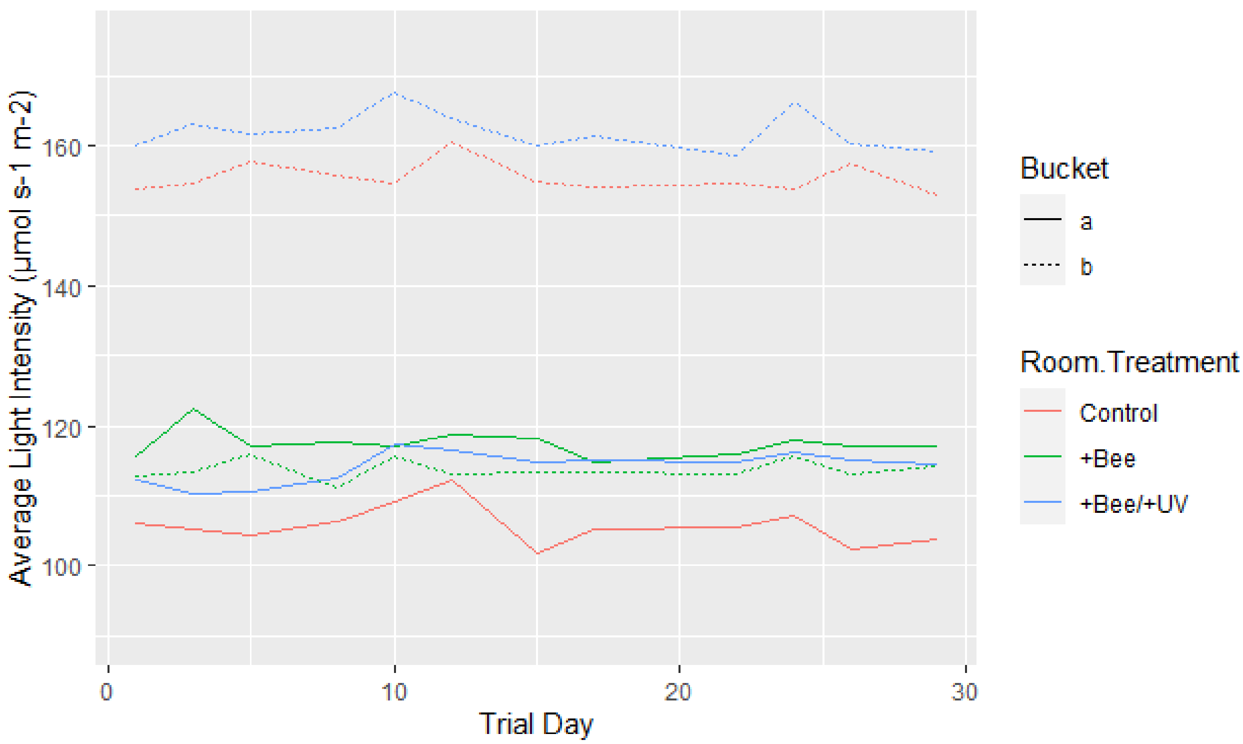
SUPPLEMENTARY FIGURE 4: **Light Intensity Summary of the Spring Trial**

Every other day, light intensity was measured at the top of each bucket using a LiCor 250 light meter. Although lights were placed the same distance away from each bucket, one bucket in both the Control and +Bee/+UV room received higher light intensity because of reflection off room walls.



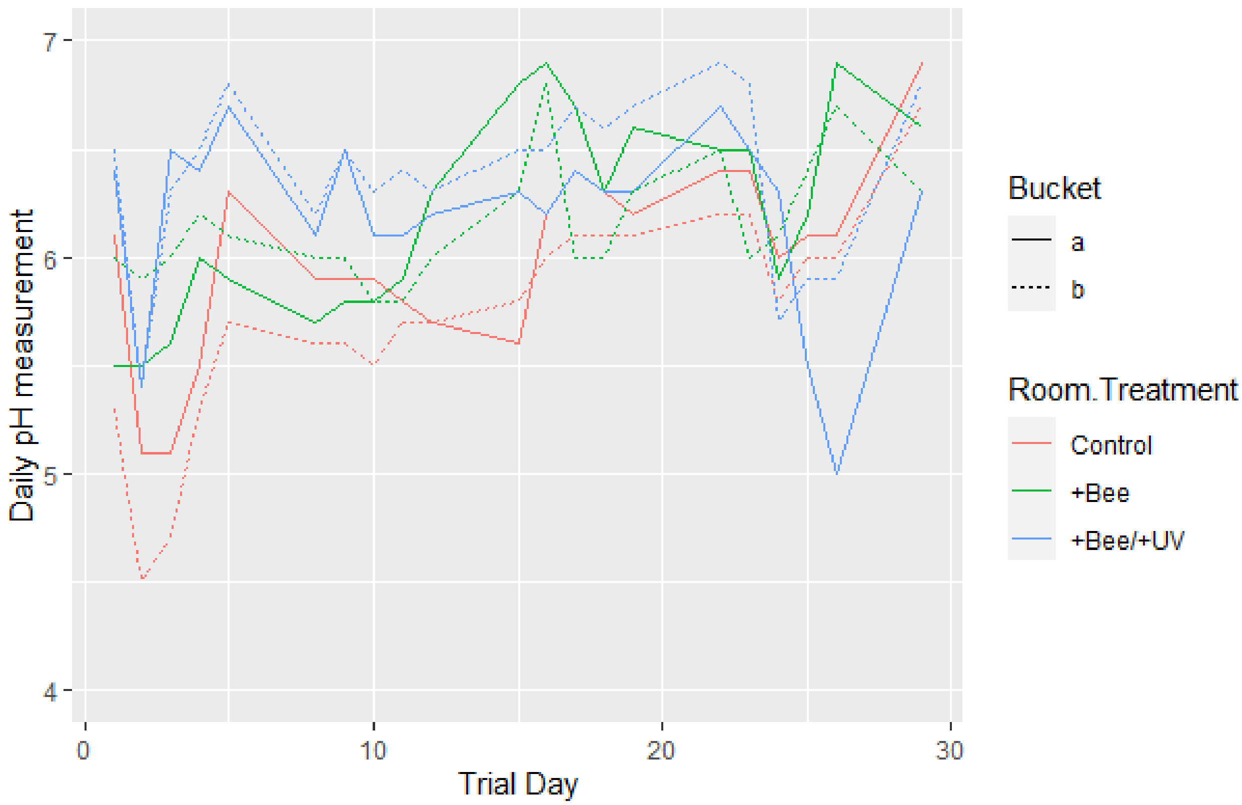
SUPPLEMENTARY FIGURE 5: **Light Intensity Summary of the Summer Trial**

Every other day, light intensity was measured at the top of each bucket using a LiCor 250 light meter. Although lights were placed the same distance away from each bucket, one bucket in both the Control and +Bee/+UV room received higher light intensity because of reflection off room walls.



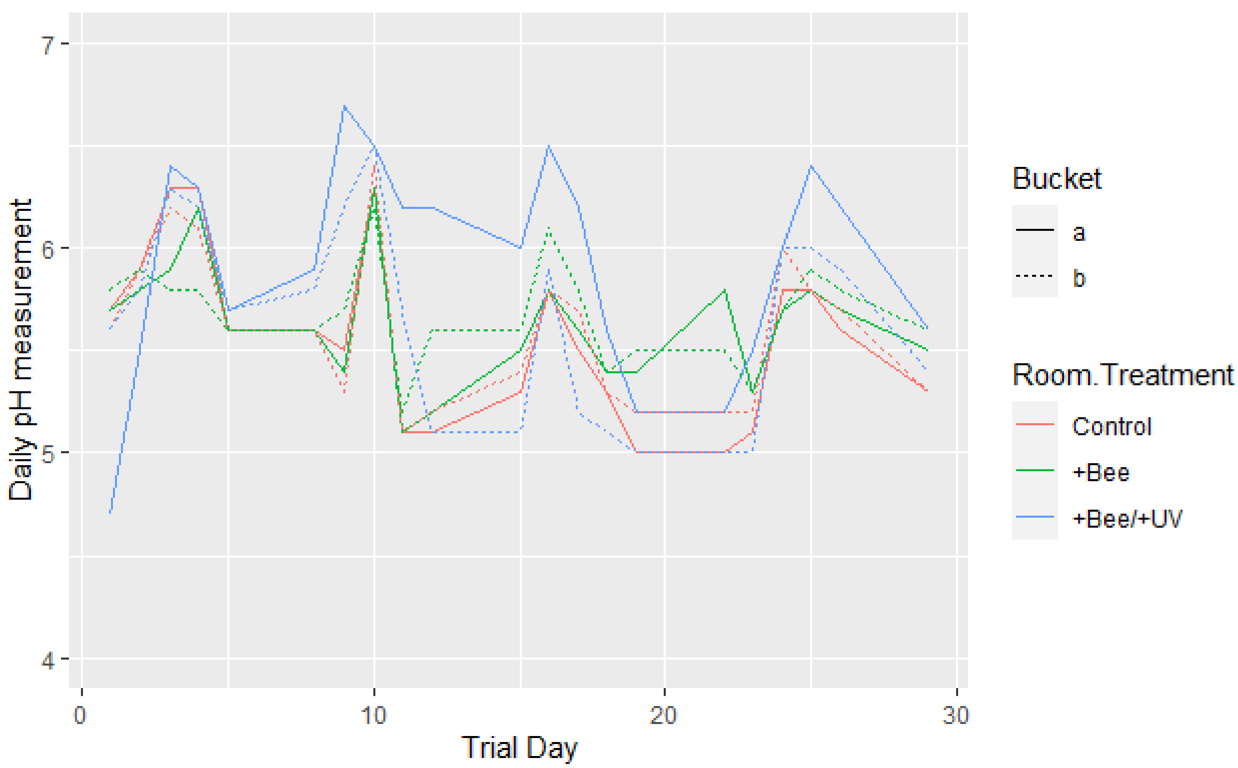
SUPPLEMENTARY FIGURE 6: **Daily pH Measurements of the Spring Trial**

Each day, pH was measured using a Blue Lab Guardian® monitor to check if any chemical additions were needed to maintain the pH between 5.1-6.9.



SUPPLEMENTARY FIGURE 7: **Daily pH Measurements of the Summer Trial**

Each day, pH was measured using a Blue Lab Guardian® monitor to check if any chemical additions were needed to maintain the pH between 5.1-6.9.

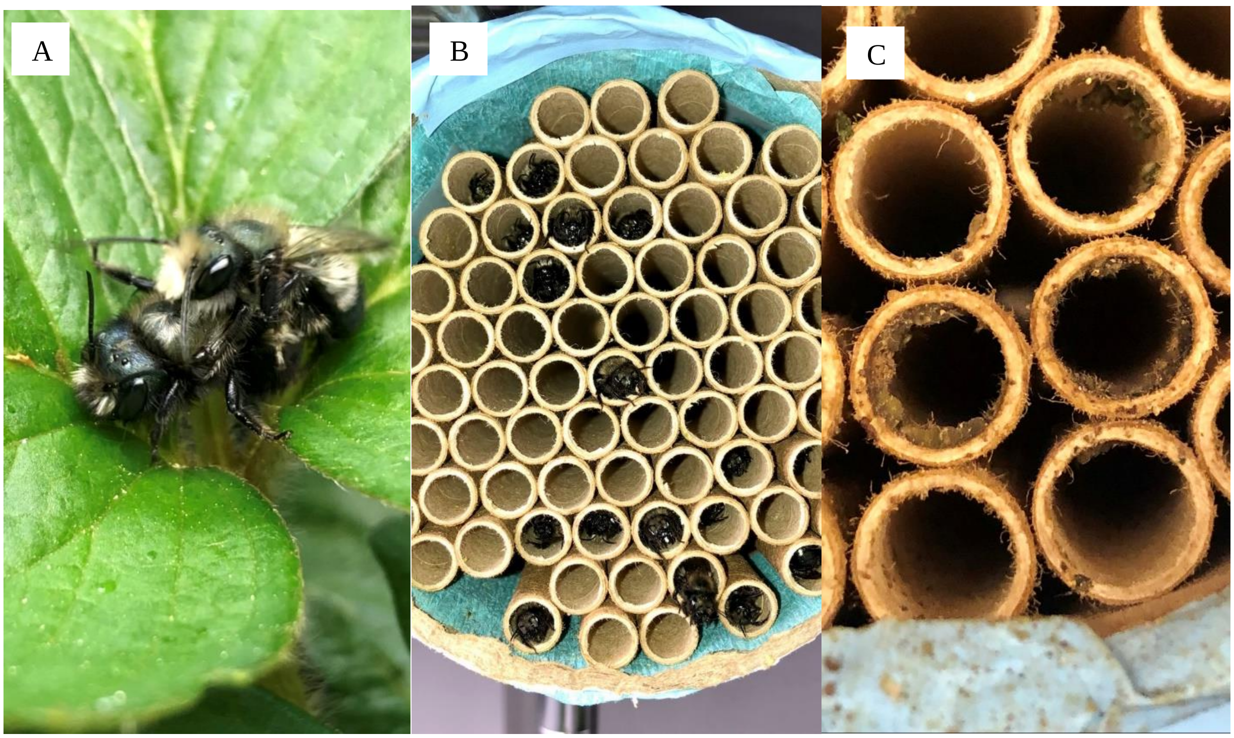


SUPPLEMENTARY IMAGES SET 1: ***Experiment Set Up***

Image A displays one bucket of strawberry plants from a bird's eye view. Image B shows the setup of the Control room and +Bee room separated by insect netting.

SUPPLEMENTARY IMAGES SET 2: **Reproductive Capability Observations**

Image A displays *O. lignaria* mating during the first few days of the spring trial. Image B shows the nesting set up and *O. lignaria* inside the tubes. Image C displays the observation of small amounts of mud around a couple tubes at the end of the summer trial.



SUPPLEMENTARY IMAGES SET 3: **Pollination Event Observations**

Image A and B are examples of the observations we made of *O. lignaria* visiting strawberry flowers.