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Strawberries (Frag X Anan) Are Bigger When Native Mason Bees (Osmia lignaria) Are Experimentally Added to Small Farms

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STRAWBERRIES (FRAG X ANAN) ARE BIGGER WHEN NATIVE MASON BEES (*OSMIA LIGNARIA*) ARE EXPERIMENTALLY ADDED TO SMALL FARMS

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ABSTRACT

STRAWBERRIES (FRAG X ANAN) ARE BIGGER WHEN NATIVE MASON BEES (*OSMIA LIGNARIA*) ARE EXPERIMENTALLY ADDED TO SMALL FARMS

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Agriculture is the cornerstone of our economy, and pollination plays a vital role in nearly all non-wind pollinated crops. Declining honey bee populations have caused a more diligent investigation into using solitary bees for crop pollination. The native, solitary Orchard Mason Bee has shown to successfully pollinate almonds and apples, but there has been little work conducted on their effectiveness as a low fruit crop pollinator. This study assesses their effectiveness as a small strawberry farm pollinator, and preferences for nesting materials. Mason bee cocoons and nest homes made with different materials (bamboo, *Phragmites*, or wood) were supplemented on a side of each of nine family-owned farms. Fruit volumes were compared between treatment and the control sides of the farms where no additions were made. Mean strawberry volumes were greater and had faster growth rates on the treatment sides. There was higher occupancy in nest homes tubes made with bamboo.

This thesis is dedicated to my parents and grandparents
who taught me to always work hard and never stop learning.

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CHAPTER 1

INTRODUCTION

Globally, humans rely on pollinators for food. In fact, 70% of 124 primary crops consumed by humans are insect pollinated (Gallai et al., 2009; Klein et al., 2007) and in Europe, 84% of cultivated crops now rely on insect pollination (Williams, 1994). The economic value of pollination globally is estimated to be $199 billion (Gallai et al., 2009). Pollinator-dependent crops are valued at about five times as much as crops not dependent on pollinators (Gallai et al., 2009). Pollinator-dependent crops, are worth about $1272/ton, on average; whereas non-insect dependent crops are worth about one-fifth of this amount ($252/ton) (Gallai et al., 2009). Thus, in crop services alone, the value of insect pollination is very high and native bees pollinate many crops, including cherry (Bosch et al., 2006; Holzschuh et al., 2012), watermelon (Winfree et al., 2007), tomato (Greenleaf & Kremen, 2006), squash, pumpkin (Shuler et al., 2005), canola (Morandin & Winston, 2005), and coffee (Klein et al., 2003).

The value of strawberry production in the U.S. is increasing annually, and was valued at approximately $2.2 billion in 2015 (National Agricultural Statistics Service & U.S. Department of Agriculture, 2016). Almost 92% of total berry production in the U.S. resulted from fresh strawberries (National Agricultural Statistics Service, 2016). While strawberries are capable of self-pollination, inbred fruits may abort, and honey bees *(Apis mellifera)* are the conventional bee used for pollination services on berry farms.

From a historical perspective, the first record of *A. mellifera* in the U.S. indicates that they arrived in bee hives that were shipped from London, England to Jamestown, VA, U.S. in 1622 (Crane, 2008). Immigrant bee populations grew in abundance and new colonies formed across the continent, through swarming. Swarming is when a honey bee colony has grown to a capacity in which part of the overpopulated colony will leave together to form a new colony with a new queen (Villa, 2004). Beekeepers collected them from the wild (Crane, 1999), which reduced the costly demand associated with shipping them from England to use for pollination and honey production. Beekeeping has grown as a profession and hobby, and there is a lucrative market for honey and wax (Popa et al., 2011), and pollination services, since bees must be trucked to orchards and farms for pollination when too few exist.

Native pollinators have been estimated to contribute up to $310 million dollars annually in pollination services to the U.S. economy, and *A. mellifera,* up to $122 million dollars annually (Allsopp et al., 2008). Populations of *A. mellifera* populations are declining in the U.S. at ~30% of hives, annually (Agricultural Research Service, 2016; Langworthy & Henein, 2009; VanEngelsdorp & Pettis, 2014). Contributing to these losses is Colony Collapse Disorder (CCD), where there is an unexplained rapid loss of worker bees to be found around the hive, dead or alive (VanEngelsdorp et al., 2009). In a study of muskmelons by Fisher and Pomeroy (1989), it was stated that poor pollination (like such that would result from population declines) caused reduced fruit set, and misshapen or asymmetrical fruit. These strawberry attributes are less preferred by consumers on ‘U-pick’ strawberry patches, where customers pick their own strawberries. The annual value of strawberry production in the U.S. is increasing every year, and was approximated at $2.4 billion in 2012, where almost 92% of the total production was from fresh strawberries (National Agricultural Statistics Service & US Department of Agriculture, 2014).

Unlike honey bees, the majority of native bees, including mason bees *(Osmia lignaria)* are solitary (Bosch & Kemp, 2001; Linsley, 1958), meaning they do not live in colonies or have structured social castes. Although they may live in close communities, they carry out daily foraging and nesting behaviors individually for the survival of their own offspring. *Osmia lignaria* are univoltine, meaning they have one brood cycle per year. They emerge from cocoons in spring as adults. Males emerge first and begin to forage for nectar in order to gain strength, while awaiting the emergence of the females, Mating ensues shortly after females wings are dry (Bosch et al., 2006; Griffin, 1993; personal observation). Once the female has collected enough semen to fertilize her eggs, she will focus on foraging, then search for a nest site. Females use nesting sites that include muddy banks, hollow wood, or man-made tubes, where females create mud walls using a mixture of local dirt and saliva. Females will then lay a single egg, which is followed by adding a small load of pollen for consumption by offspring at a later time. Then another mud wall will be formed next to the pollen reserve. Females repeat this process, creating multiple cells, which are ultimately capped with mud. Females have the ability to control the gender of each egg through fertilizing, or lack thereof. Female eggs are laid in the deepest cells first, and the rest are male, typically laid in a ratio of about four females to six males (Bosch & Kemp, 2000; Rau, 1937). Once a nest tube is filled, females move to an adjacent nesting tube or nesting tube close to the original nesting tube. Adult female *O. lignaria* can be seen foraging until the end of spring, when these females then die naturally. Eggs laid throughout the spring transform into larvae and emerge as adults in the subsequent spring (Bosch & Kemp, 2000; Rau, 1937).

The early spring emergence of *O. lignaria* makes them ideal candidates for pollinating strawberries, which are an early spring crop. These *Osmia* are effective pollinators of fruit trees and almonds trees (Bosch & Kemp, 2000; Bosch et al., 2000). However, little work has been conducted to determine whether they will successfully pollinate other crops, including strawberries, on farms. Many of Virginia's crops are primarily pollinated by non-native honey bees. There would be a dramatic economic loss to be incurred with a complete disappearance of the honey bee, impacting the health of crops, those in the agricultural business, and on the end consumer as well (Allsopp et al., 2008; Losey & Vaughn, 2006).

Without the addition of bee pollination, the quality of the remaining crops would suffer. This could result in producers having a reduction in fruit quality, which would reflect in profits. It could also cause an increase in price to the consumer if harvests become smaller, and more in demand. There is a dire need for more research on native pollinators. Importing honey bees from other countries, or paying to loan bees from an apiary would result in higher costs to the producer. Mason bee cocoons are available naturally, and also at reasonable prices through dealers. This allows farmers to buy mason bee nest boxes, or create their own, in order to help attract these bees.

The goals of this study were to determine whether *O. lignaria:* a) could be used successfully on small farms to actually pollinate a berry crop, b) could improve strawberry yield, b) would colonize nest boxes placed on farms, c) would demonstrate a preference for a particular type of nest home materials over other materials. Additionally, identifying whether there were costs and benefits of importance that differed between nest home materials was assessed.

CHAPTER 2

METHODOLOGY

EXPERIMENTAL DESIGN

The study spanned two field seasons in the spring and summer of 2014 and 2015. Nine strawberry patches on small farms in Pungo, VA, which were predominantly consumer-harvested, were used. In the first field season (2014), one half of each patch was considered the treatment, and mason bee cocoons were added. The other half of the patch was a control. These halves were less than the typical flight radius of a mason bee, which left a ‘no fly’ zone in between the treatment and control (Fig. 1). Experimental sides were also supplemented with nesting habitat and control sides were not (Fig. 1). Mason bee nest homes were spaced approximately seven meters apart from one another and mounted about one meter in distance from the outermost strawberry row (Fig. 1).

At the start of the 2014 spring field season, 90 early strawberry flower blooms that had emerged but were not yet open were randomly tagged on the control and treatment sides of each berry patch. Each plant was marked in the field uniquely with a wooden craft stick as well for easy identification as plants grew. Bloom stems were tagged with a cable loosely tied around the stem, and growth of the strawberry was monitored throughout the season. In the 2015 field season, blooms were tagged on three different farms. Berry size was measured throughout the season until the strawberries ripened or were picked.

STRAWBERRY MEASUREMENTS AND ANALYSIS

Starting in April in both 2014 and 2015, measurements were recorded for each tagged strawberry until they were bright red on most of the surface or were picked by consumers, which was the beginning of July in 2014, and the end of June in 2015. Height (from base of sepals to distal tip of strawberry) and width (taken at widest point of strawberry) (Liming & Yanchao, 2010)(Fig. 3) were the quantitative data collected. The conical area formula was used to assign a volume to each strawberry for each recorded measurement from the height and width data.

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

A nonparametric ANCOVA, Quade's Test (Quade, 1967) was used to calculate the effect of mason bee addition, the concomitant variables, and their interactions. Patch size (acres) and duration of growth (days elapsed between bloom and ripened strawberry) were used as the concomitant variables in the test of fruit volume.

Growth rate of each strawberry was calculated as follows:

$$Growth\ rate = \frac{final\ fruit\ volume}{number\ of\ days\ to\ ripen}$$

Because data for growth rate were also not normally distributed, a Quade's test was performed to calculate the effect of mason bee addition, the concomitant variables, and those interactions. Patch size and duration were also used as concomitant variables in the Quade's testing of growth rate.

SYMMETRY ANALYSIS

Fruit symmetry was initially qualitatively recorded (symmetrical or asymmetrical) in the field and one patch was quantified using two different imaging programs. Using a set of strawberry photos taken on a single day prior to ripening, symmetry was quantified. Images were taken in the same format, from a lateral view. ImageJ (NIH, MD, USA), an open source image processor, was used to trace an outline around the strawberry in each photograph, which returned a total area measurement in pixels, as well as the pixel coordinates of the berry centroid. Next, a line was drawn on the image from the center of the stem, through the centroid, creating a midline (Fig. 4). The area of the strawberry was measured independently, on each side of the midline. The absolute value of the difference in pixels between half of the total area (bilateral symmetry) and the area of each side of the strawberry was divided again by half of the total area. That number was calculated as the 'asymmetry value':

$$Asymmetry\ value = \frac{\left|\left(\frac{total\ \ area}{2}\right) - area\ of\ sideA\ or\ B\right|}{\left(\frac{total\ area}{2}\right)}$$

The greater of the two measures was recorded for each strawberry for a total of 28 strawberries from the control and 34 from the experimental plot. Normality assumptions were also not met for these data, so a nonparametric Mann-Whitney U test was used to compare asymmetry values between strawberries from the MB- and MB+ sides.

These photographs were also run through a new program called Ka-Me: a Voronoi image analyzer (NCBI, MD, USA). This program takes user-generated points on an image (here a dot on each achene or seed on a lateral photograph of each berry), and performs a Delauney triangulation, with which it connects the centers of the triangulations to create a Voronoi diagram (Khiripet et al., 2012). The Voronoi diagram displays a map of contiguous polygons based on the distances between generator points. The resulting output displays the total number of polygons found for each type of polygon (5 angles (pentagon), 6 angles (hexagon), etc.), and these were recorded in a histogram as well as the number of generator points marked, the mean number of internal angles, and a skewness value from the histogram (Fig. 5). Hexagons are a type of symmetric structure common in nature. Aside from their commonality as a polygon in Voronoi tessellations, they are also very stable as a pattern of cells. With the introduction of more hexagons, stability increased (Lucarini, 2008). As a pattern is disturbed, the number of pentagons and heptagons around the hexagons will be become disproportionate, with pentagons becoming more prevalent than heptagons (Tanemura et al., 1991). In order to quantify this disproportion around the hexagons, the skewness value was used. Because skewness measures the asymmetry of the probability curve, we could assume that a skew value of zero indicated proportionate frequencies of pentagons and heptagons surrounding the hexagons. Regardless of the direction of the skew, further deviation from zero indicated asymmetrical polygon counts and increased polygon pattern disturbance. The skewness values for the strawberries on the MB+ and MB- sides were compared using a nonparametric Mann-Whitney U test, since normality assumptions for these data were not met.

If a predominantly stable hexagonal pattern is present, this leads one to conclude they have been more fully pollinated, which the farmers had noted makes a more symmetrical fruit. This is supported by a study done by González et al. (2006), which showed increased adhesion of pollen during hand pollination of cherimoya (*Annona cherimola*) resulted in more symmetric fruit.

NEST HOMES AND COCOONS

Four types of mason bee homes were constructed in the winter of 2013. The first two types were made from recycled plastic jugs filled with tubes made from ~15-20cm long segments of either bamboo or *Phragmites australis* subsp. *australis* found growing regionally, but according to city officials, had not yet been sprayed with herbicides (Fig. 2). Homes with bamboo contained 60 tubes with a radius as close to the range of 8-9.5mm as possible. Homes with *Phragmites* contained 90 tubes of the same. The third and fourth home type were constructed from untreated pine lumber, and each box had approximately 23-15cm deep holes drilled into them, each, a radius of 9.5mm (Fig. 2). The block style of wooden nest home was 3 blocks of wood glued together with wood glue, and had 8- 15cm tubes drilled directly into each block of wood, totaling 24 tubes per home. The second type of wooden nest home used a tray design with 5-7 half circles drilled 1cm apart into 5- 20cm pieces of wood ~~up~~. When these 5 pieces of wood were stacked on top of each other, the half circles lined up to form tubes. The tubes in wooden nest homes had a parchment paper liner to help prevent the movement of pathogens between tubes, and to aid in the removal of cocoons after the season was over.

The number of bee cocoons deployed was based upon the acreage of the patch used, such that the density of emerging bees would be equal to 630 per acre. Since this type of work has never been conducted on berry patches before, estimates for deployment were based upon apple orchard work (Torchio, 1985). In 2014, the largest strawberry patch, "HF2" (1.59 acres) received 44 mason bee nest boxes made of recycled milk jugs filled with nesting tubes constructed of either bamboo or *Phragmites* (22 jugs of each type). A total of 22 *Phragmites* homes containing a total of 1,980 tubes were set out on the farm.

Each cocoon was placed inside an individual tube hole in the nest box. The number of nest boxes per strawberry patch was calculated by taking the number of cocoons need per patch and dividing that by 23, the approximate number of tubes in our wooden nest homes. Nest home inspections were done at the end of each season to determine the number of mud-capped tubes of mason bee eggs and to check for evidence of parasitic wasps.

CHAPTER 3

RESULTS

FRUIT VOLUME

Mean fruit volumes (mm^3) had a leptokurtic distribution and did not meet assumptions for parametric testing. For the nine strawberry patches included in this study, there was a significant difference in mean fruit volume between the control (MB-) (2919.27 ±151) and treatment (MB+) (4418.72 ±207) side (Quade's Test, $F_{(1,762)}$= 21.836, $p < 0.001$) (Fig. 6). Given these strawberry patches varied in acreage, a linear regression was performed to determine whether patch size is a predictor of (or explains any of the variance in) mean fruit volume. The regression equation was not significant ($F_{(1, 7)}$= 0.00074, $p = 0.979$), with an $R^2 < 0.001$ (Fig. 7) meaning berry size did not vary with patch size.

There was also a significant difference in mean fruit volume in both treatment and control sides combined between 2014 (2,593.42 ±112) and 2015 (6,592.16 ±298) (Mann-Whitney U test, Z= -13.103, $p < 0.001$). However, year could not be used as a covariate without violating the assumption of random concomitant variables, so Quade's test was performed for each year separately. In 2014, there was a significant difference in mean fruit volume between control (1,920.40 ±109) and treatment (3,210.5 ±183) sides ($F_{(1,552)}$= 21.702, $p < 0.001$). In 2015, there was also a significant difference in mean fruit volume between control (5,440.2 ±354) and treatment (7,744.09 ±454) sides ($F_{(1,208)}$= 12.915, $p < 0.001$).

Mean fruit volume was also greater for the treatment patches relative to the control for 8 out of the 9 strawberry patches (Table 1). However, an ANOVA was performed on the NEF where the mean fruit volume was greater on the control side, because that subset of data was normally distributed. The results of the ANOVA indicated that the difference in the mean fruit volume from this particular patch, was not significant ($F_{(1, 70)}$= 0.062, p= 0.805).

GROWTH RATE

In looking at the possibility of the addition of mason bees hastening up the ripening process, a Quade's test was performed on the non-normally distributed data set, comparing the growth rates (mm^3/day) (as described in methods) of each patch side, while taking the patch size and duration into account as concomitant variables. There was a significant difference in the growth rates between the control (151.62 ±7) and treatment (219.97 ±10) sides ($F_{(1,762)}$= 21.342, p< 0.001) (Fig. 8).

A linear regression was performed to determine whether the size of the strawberry patch impacted fruit volume. The regression equation was not significant ($F_{(1, 7)}$= 0.092, p= 0.771), with an R^2 of 0.013 (Fig. 9) indicating that berry volume is not impacted by patch size.

Again, a Mann-Whitney U test performed on the non-normal data set to determine if there was a difference in simply the overall growth rates of each year. The test revealed there was a significant difference of growth rates between year 2014 (163.25 ±7) and 2015 (249.16 ±11) (Mann-Whitney U test, Z= -8.049, p< 0.001). A Quade's tests was then used to evaluate each year separately, using the same concomitant variables, patch size and duration. In 2014, there was a significant difference in growth rates between the control (127.6 ±7) and treatment (195.96 ±12) sides ($F_{(1,552)}$= 21.739, p< 0.001). In 2015, growth rates differed significantly between the control (212.27 ±14) and treatment (286.04 ±17) sides ($F_{(1, 208)}$= 11.615, p= 0.001).

The mean growth rate was higher on the treatment side than the control side for 7 out of the 9 strawberry patches (Table 2). An ANOVA was performed on one of these two patches where the growth rate on the control side was larger, and assumptions were met. The ANOVA results indicated the difference between the sides in that strawberry patch were not significant ($F_{(1, 70)}$=0.617, p= 0.435). For the second strawberry patch showing a reverse effect with growth rate, the data were not normally distributed, so a Mann-Whitney U test was performed and also indicated that there was not a significant difference in the growth rates between the patch's two sides (Mann-Whitney U test, Z= -1.423, p= 0.155).

FRUIT SYMMETRY

The ImageJ method of analyzing symmetry resulted in data with a non-normal distribution, so a Mann-Whitney U test was performed to determine if there was a difference in the "asymmetry values" of each side. The test showed a significant difference between the control and treatment sides of the patches. The mean asymmetry value was larger on the control sides (0.0172±0.0107) than on the treatment sides (0.0095 ±0.0044) (Mann-Whitney U test, Z= -2.659, p= 0.008).

The data analyzed from the photos that were run through the Ka-Me program also showed a significant difference in skewness value between the control and treatment sides (Mann-Whitney U test, Z= -3.395, p= 0.001). The mean skewness value was larger on the control sides (0.1423 ±0.1922) than on the treatment sides (0.0184 ±0.0867). A skewness value closer to zero is considered the most symmetrical.

COLONIZATION RATES AND NEST BOX PREFERENCES

A total of 22 bamboo homes containing a total of 1,320 tubes were set out on Patch 5, the biggest patch. Of the 1,320 bamboo tubes available, 19.5% of tubes (258 of 1,320 available tubes) were mud capped. Of these, 1.2% (3 tubes) were parasitized by parasitic wasps. One home (4.6%, 1 of 22 homes) was damaged from the tubes spilling out of the home.

A total of 22 *Phragmites* homes containing a total of 1,980 tubes were set out on the HF2 patch. Of the 1,980 *Phragmites* tubes available, only 4.8% of tubes (96 tubes) were mud capped, of which 6.3% (6 tubes) were parasitized. Seven of these homes were damaged, knocked over, or with tubes that had spilled out (31.8%, 7 of 22 homes).

The other five strawberry patches that received mason bee cocoons in 2014 received wooden nest homes. There were 57 wooden nest homes (20 block type, 37 tray type) that were allocated to the remaining treatment patches in the first year, based on the patch size. Of these 57 wooden nest homes, there was a total of 1,468 available tubes, and only 54 tubes were mud capped (3.7%). Of these 54 mud capped tubes, 16 tubes were parasitized (29.6%). The 20 wooden block nest homes had a total of 480 tubes, 23 of which were mud-capped (4.8%), and none were parasitized. The 37 wooden tray nest homes has a total of 988 tubes, 31 of which were mud-capped (3.1%).

Of those 31 mud-capped tubes in the tray nest homes, half were parasitized (51.6%, 16 of 31 mud-capped tubes).

The bamboo and *Phragmites* nest homes were better used by the mason bees with 10.7% of all tubes mud capped (354 of 3,300 tubes), compared to only 3.7% of wooden home tubes being mud capped (54 of 1,468 tubes). In terms of parasite preference, the wooden nest homes had more mud capped tubes parasitized (29.6%, 16 of 54 tubes) than those of the recycled jugs containing either Bamboo or *Phragmites* (2.5%, 9 of 354 tubes).

A Quade's test was performed and showed a significant effect of nest home material (bamboo, *Phragmites*, and wood) on the frequency of mud-capped tubes relative to uncapped tubes per nest home, after controlling for the size of the patch on which each nest home was located ($F_{(2,98)}$= 11.235, p< 0.000). A Quade's test of the effect of nest home material on the frequency of parasitized tubes relative to mud-capped tubes per nest home was performed, and showed no significant effect after controlling for the size of the patch on which each nest home was located ($F_{(2,54)}$= 0.153, p= 0.858).

Data were analyzed again, breaking the nest home material down into the four types of nest homes (bamboo, *Phragmites*, wooden blocks, and wooden trays). There was a significant effect of nest home type on the frequency of mud-capped tubes relative to uncapped tubes per nest home, after controlling for the size of the patch on which each nest home was located ($F_{(3,97)}$= 9.338, p< 0.000). A Quade's test of the effect of nest home type on the frequency of parasitized tubes relative to mud-capped tubes per nest home was performed, and showed a significant effect after controlling for the size of the patch on which each nest home was located ($F_{(3,53)}$= 3.488, *p*= 0.022).

CHAPTER 4

DISCUSSION

The Orchard Mason Bee is an alternative option for sustainable pollination. *Osmia* cocoons were purchased for an average of $0.82 per cocoon when bought in bulk. Protective gear (suits and smokers) are unneeded expenses for working with these mild-mannered, non-colonial bees. The work involved with upkeep is less than that for honey bees, because unlike honey bees who are active all year, *O. lignaria* are actively foraging in spring. The rest of the year is spent by the eggs developing into adults within the cell to emerge the next spring (Griffin, 1993). There is no refilling water and extra food throughout the colder months. A farmer could continue to buy new cocoons and nesting material each year, which may be simpler. They could also choose to check for parasitism while the nest homes are out, and bring cocoons or tubes in to a protected outdoor space or refrigerated storage during summer in order to use again the next year. This would take more time and effort, but would allow a farmer to potentially reduce the need to buy additional cocoons the next year. The steady yearly decrease in need to purchase cocoons from a supplier should eventually allow the farmer to have enough cocoons from the previous year. However, the sustainability of this is not yet certain, as parasitism has a large impact on the number of cocoons available to the farmer for the following year.

The benefit of these insects being able to successfully pollinate and improve the value of strawberries indicate economic value in using these bees for fruit crop production. Based on the latest reports of national strawberry production by the National Agricultural Statistics Service (2014), one acre of strawberries would have an average yield of 523cwt (hundredweight), and each cwt would return $80.40. That would mean on average, for every acre of strawberries planted, there is a little over $42,000 in potential profit. If mason bees were added to this 1 acre patch, approximately 630 cocoons would be needed to mimic our study. If each cocoon cost an average of $1.47 (or an average of $0.82 when purchased in bulk quantities), the cost would be about $926 in cocoons/acre ($517 for bulk orders). Tubes for these homes cost an average of $0.52 per tube. This study also used approximately 630 tubes per acre, which would cost $328 per acre. If this addition caused the production to increase by 66% (as that is the average increase in fruit volume across the farms in this study), that would increase the yield per acre to 868.18cwt, returning just over $69,800 in potential profit. This $27,800 increase in revenue per acre far exceeds its cost of $1,254.

Although all farms in this study had honey bee hives in their vicinity, the honey bees could freely access each entire farm and had a foraging radius well beyond individual patch sizes. Thus, the addition of mason bees contributed to pollination services and impacted final fruit size. Mason bees were repeatedly observed collecting pollen, foraging on berry flowers frequently and moving between strawberry flowers – putatively allowing for outcrossing. Higher mean fruit volumes on treatment plots likely suggest a noticeable effect from the addition of mason bees, given our behavioral observations aimed at determining whether mason bees are pollinating crops and whether they incite honey bees to increase pollination (manuscript in prep). Further studies of the possible synergistic effect of the honey bees and/or other non-*Apis* bees are relevant and warranted. A synergistic effect has been shown in numerous studies to increase fruit set and pollination efficiency (Brittain et al., 2013b; Garibaldi et al., 2014; Klein et al., 2007; Rogers et al., 2014), but our preliminary observations do not suggest additional pollination by honeybees and do suggest pollination by mason bees.

There was a preference for the bamboo and the *Phragmites* nest homes over the wooden nest homes. The preference for the bamboo and *Phragmites* segments used to make the nest tubes are a clear reflection of the materials that the *O. lignaria* would utilize in nature. The sturdier and usually larger diameter bamboo stalks show in the bees' preference for them. Utilizing these invasive species as nesting materials is a time-consuming process (Fig. 10), but very ecologically friendly. Parasitization could have been lessened if nest homes had been brought in sooner than planned, once evidence of it had begun to appear.

The wooden nest homes were easier to make, and the preference for tubes drilled into wooden blocks over stacked trays of tubes only enforce the ease and adaptability of these mason beekeeping practices. Although trays were less preferred by *O. lignaria*, they were easier to mass produce in large quantities. In future practice, commercial farming operations may benefit from collecting homes post colonization and storing the cocoons away from the threat of parasitism and severe weather or catastrophic events. Cocoons are inaccessible and relatively protected inside bamboo and *Phragmites* tubes and even wooded blocks, until spring when the new adults emerge.

In addition to parasitism, nest boxes on farms were also utilized by wasps, ants, and spiders. Potter wasps (Eumeninae), such as the Four-toothed Mason Wasp (*Monobia quadridens*) and *Ancistrocerus* spp., and thread-wasted wasps (Sphecidae) such as the Black and Yellow Mud Dauber (*Sceliphron caementarium*) and grass-carry wasps (*Isodontia spp.*) were the most frequently seen using holes.

Some problematic issues arose during this work that could be corrected, including communication issues between researchers, farmers and farm hands to prevent changes to nest home layouts or other farming practices that may disturb the study.

CHAPTER 5

CONCLUSIONS

This is the first study to show that mason bees are valuable for commodities other than tree crops, can successfully be added to small farms to pollinate berry flowers and do improve the value of strawberries. If farmers choose to supplement their farms with mason bee cocoons and habitat each year, fewer *Osmia* will need to be bought to maintain an ideal number of pollinators, as the offspring from the previous generation will start to multiply each year. This should, in theory, lead to a mostly self-sustainable practice.

There is also recently completed work that will demonstrate the use of *O. lignaria* in the pollination of berries in greenhouses. This study has since began work looking into the synergistic effects of mason bees and other pollinators on pollination. There is also research started on determining the ideal density of mason bees for pollination. In sum, *O. lignaria* show great promise for use in pollination services beyond orchard trees and were successfully introduced on small farms to show a measureable beneficial effect on a strawberry crop across two years.

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APPENDIX A

TABLES

Table 1: Mean fruit volumes (mm^3) with standard error for both control (MB-) and treatment (MB+) sides on each patch. Patches are listed alphabetically.

Farm	MB- Mean		MB+ Mean		Year
CBP	1514.80	(±200)	5270.69	(±877)	2014
CFM1	1329.46	(±218)	2434.22	(±414)	2014
CFM2	1998.90	(±308)	2267.07	(±361)	2014
CNO	4949.7	(±486)	9797.48	(±747)	2015
HF1	6887.46	(±748)	9191.27	(±804)	2015
HF2	3282.08	(±423)	4630.23	(±356)	2014
NEF	4550.18	(±525)	4380.94	(±435)	2015
VFP1	1254.73	(± 97)	1940.51	(±144)	2014
VFP2	2575.68	(±262)	3298.24	(±457)	2014

Table 2: Mean growth rates (mm^3/day) with standard error for both control (MB-) and treatment (MB+) sides on each patch. Patches are listed alphabetically.

Farm	MB- Mean		MB+ Mean		Year
CBP	85.32	(± 13)	360.08	(± 67)	2014
CFM1	92.39	(± 16)	176.52	(± 32)	2014
CFM2	152.04	(± 23)	127.32	(± 22)	2014
CNO	167.61	(± 16)	319.00	(± 26)	2015
HF1	288.86	(± 31)	382.97	(± 34)	2015
HF2	148.46	(± 17)	224.79	(± 17)	2014
NEF	183.36	(± 21)	162.45	(± 16)	2015
VFP1	89.62	(± 7)	138.61	(± 10)	2014
VFP2	208.95	(± 21)	259.27	(± 43)	2014

APPENDIX B

FIGURES

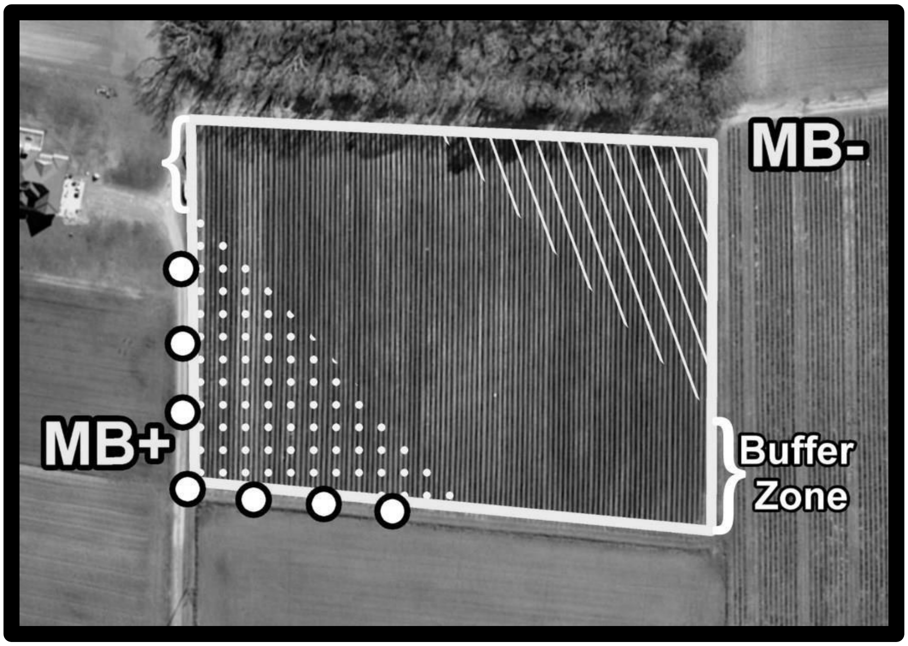

Figure 1: Layout of strawberry patch. A portion of the patch received mason bee cocoons and nest boxes on the treatment side (MB+), while the control side (MB-) was not manipulated.

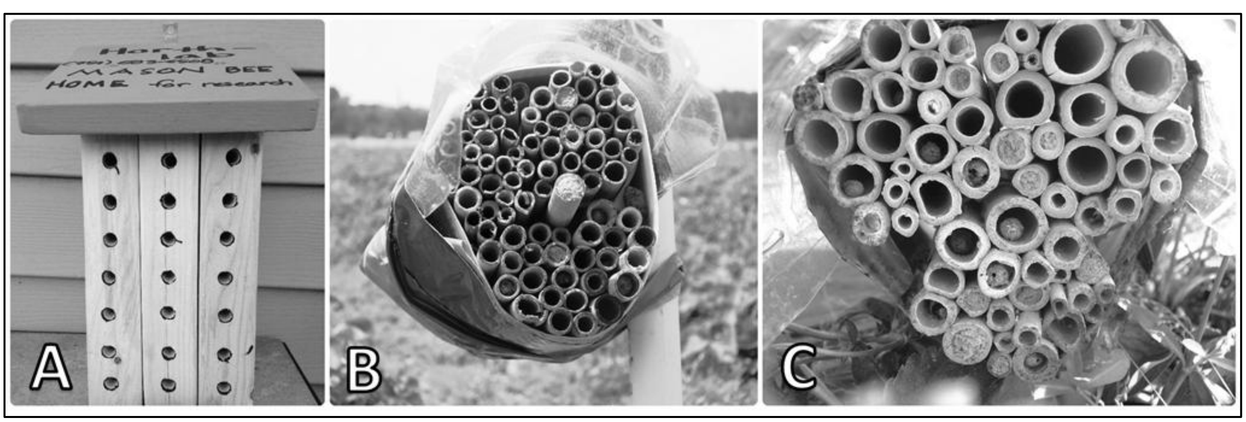

Figure 2: Types of nest homes used. A) Wooden nest box; B) Recycled jug with *Phragmites* tubes; C) Recycled jug with Bamboo tubes.

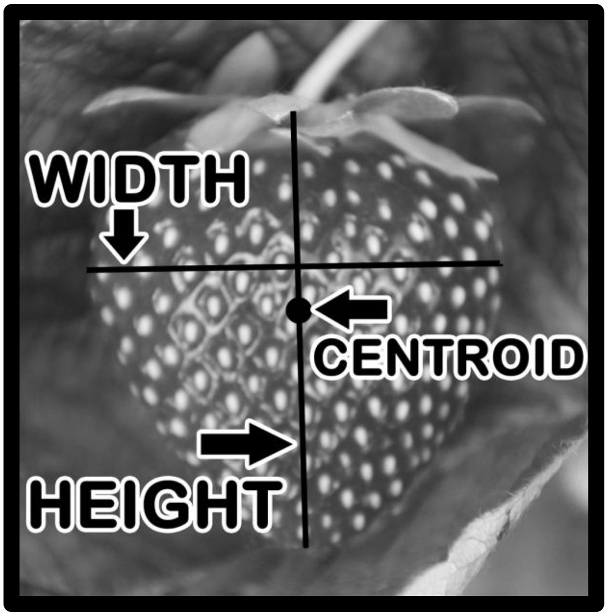

Figure 3: Description of the field measurements taken in order to calculate fruit volume. Height is measured from the center of the calyx to the distal tip of the strawberry. Width is measured at the widest point of the strawberry side.

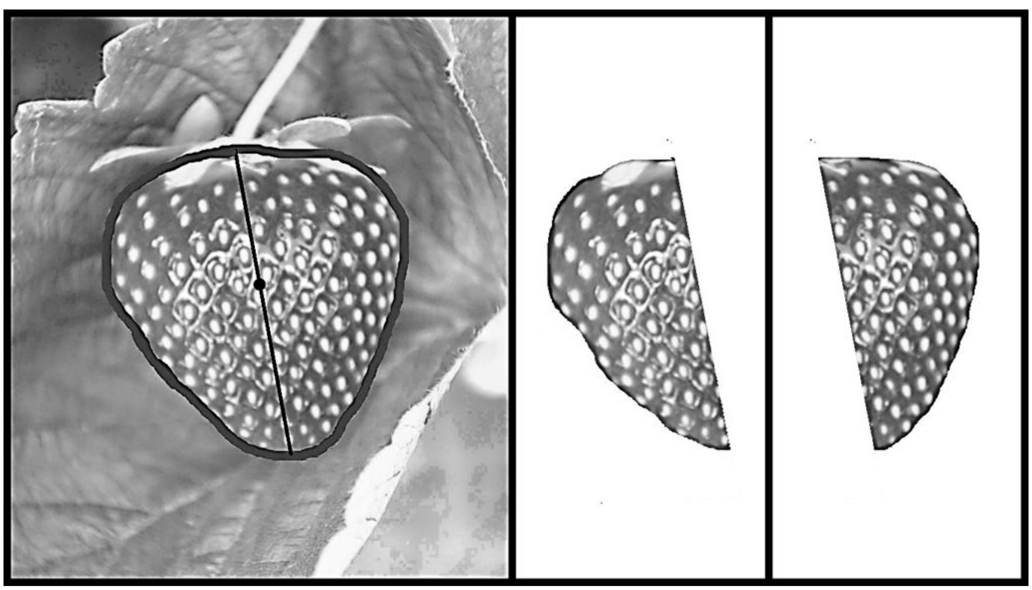

Figure 4: Description of method to calculate asymmetry value in ImageJ program. ImageJ measurements were taken by outlining the strawberry to find its centroid, then drawing a line from the center of the stem through the centroid, bisecting the strawberry into two parts. The area of each side was compared against half of the total area of the strawberry. The absolute value of the difference was considered its asymmetrical value.

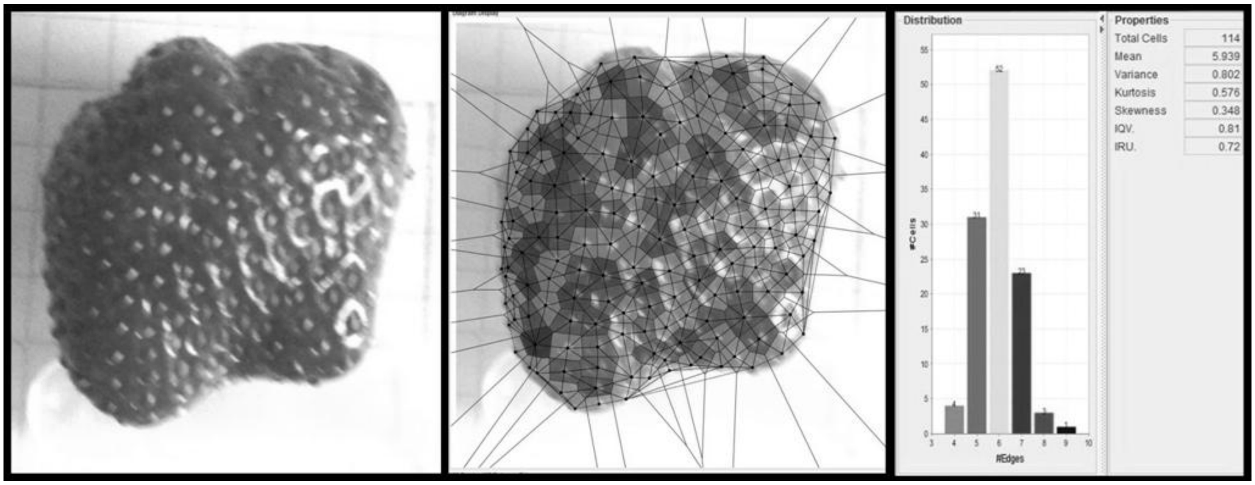

Figure 5: Stages of Ka-Me program output. Asymmetric strawberry (left); screenshot of Ka-Me program with Voronoi tessellations plotted on strawberry, where the shading of the different cells indicate the type of polygon it is (middle); screenshot of the program's output (right).

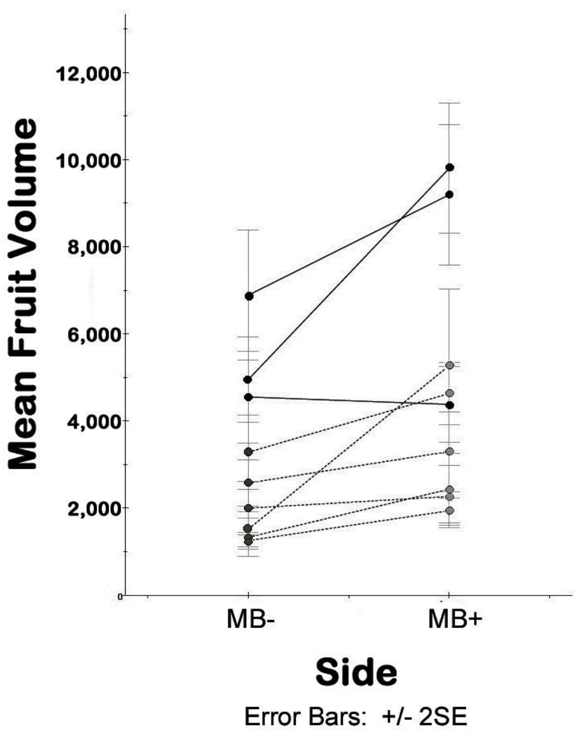

Figure 6: Mean fruit volumes (mm^3) for all nine strawberry patches across both years. Dashed lines indicate 2014; solid lines indicate 2015.

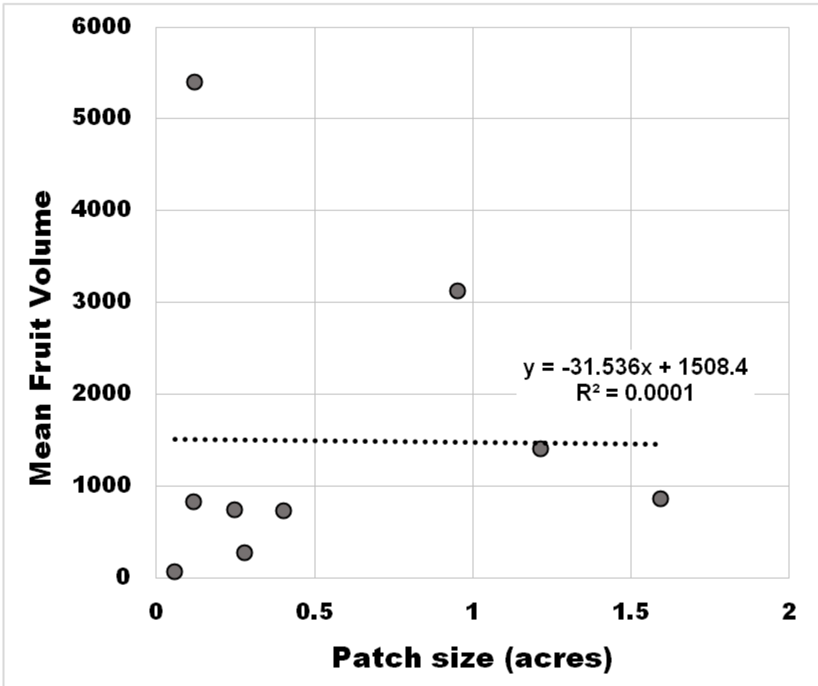

Figure 7: Linear regression demonstrating that patch size accounts for nearly none (0.01%) of the variation in mean fruit volumes.

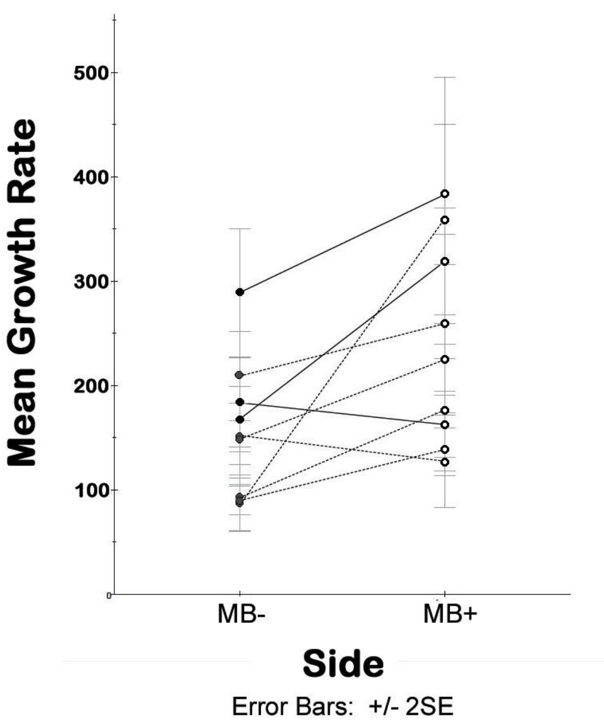

Figure 8: Mean growth rates (mm^3/day) for all nine strawberry patches across both years. Dashed lines indicate 2014; solid lines indicate 2015.

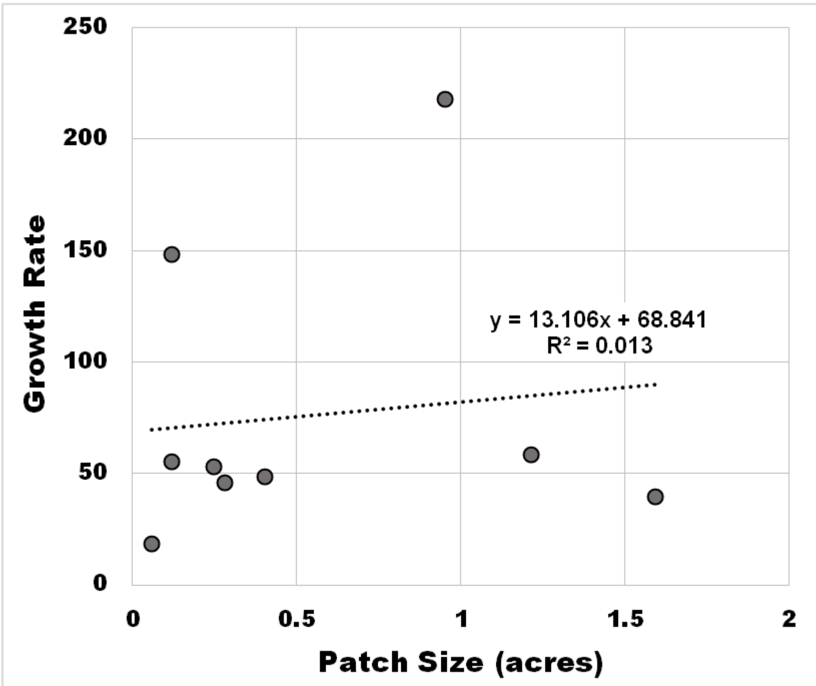

Figure 9: Linear regression showing patch size accounts for only 1.3% of variability in growth rate.

Figure 10: Groups of volunteers worked to assemble the needed amount of nest homes from recycled plastic jugs, *Phragmites*, and Bamboo stalks.

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Virginia State Beekeepers, 2013 – 2016

The word processor for this thesis was Microsoft Word.