THE DEVELOPMENT OF BEST MANAGEMENT PRACTICES OF COMMERCIAL BUMBLE BEES (HYMENOPTERA: APIDAE) ON HORTICULTURAL CROPS IN DELAWARE

by

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ABSTRACT

Commercial bumble bees first became available in the 1980s in the Netherlands. Since then commercial bumble bees have grown in popularity amongst growers as honey bee populations continue to decline. Currently, bumble bees are the leading pollinator in the greenhouse tomato crop, and are so advantageous that countries without the ability to import commercial bumble bees are at an economic disadvantage on the world's stage. Much remains unknown, however, on their efficiency in horticultural crops and how to best manage these pollination units for optimal use. The goal of this project was to use scientific experimentation to develop the Best Management Practice document for growers in Delaware on how to best use these units in horticultural crops. Chapter 1 divulges on the scientific experimentation conducted during the 2011 and 2012 growing season on these pollination units in strawberry, watermelon, and pickling cucumber crops in Ken and Sussex counties in Delaware. Chapter 2 is the developed Best Management Practice document written for the sole use of Delaware growers.

Chapter 1

USING THE COMMERICAL BUMBLE BEE, *BOMBUS IMPATIENS* Cr. (HYMENOPTERA: APIDAE) ON HORTICULTURAL CROPS IN DELAWARE

1.1 Introduction

Each year in the state of Delaware over 4,000 acres of cucumbers are planted and harvested at a value of 4.5 million dollars, roughly 3,000 acres of watermelon are planted and harvested and are valued at over 10 million dollars and strawberries alone bring close to a half a million dollars into the state (USDA 2007). All of these crops rely upon the pollination services provided by insects. The 'western' honeybee (*Apis mellifera*) is arguably the most economically valuable pollinator of agricultural crops worldwide (Free 1993, Robinson et al. 1989) and the most widely used and economically important managed pollinator in Delaware.

1.1.1 Honey bee problems and declines

The economic value of honey bees nationwide is $17 billion (Calderone, 2012). Unfortunately, honey bee populations have been declining over the past decade due to their susceptibility to numerous pests and pathogens which has had profound negative effects on their ability to over winter and therefore build up populations in the spring when many crops start to bloom (Artz and Nault 2011, Stanghellini et al. 1997, 1998, 2002, Stubbs and Drummond 2001, vanEnglesdorp et al. 2007, 2008). This decrease in managed pollinator populations during the blooming

period of many important crops has growers and pollination ecologists concerned and looking to alternative pollinators.

1.1.2 Bumble bee biology

To help combat pollinator shortages and meet the pollination requirements of their crops, researchers and growers are investigating the effectiveness of native pollinators in larger agricultural settings. A common native pollinator in Delaware is the Common Eastern Bumble Bee (*Bombus impatiens*). The behavior, physiology and morphology of bumble bees make them ideal pollinators because of the speed at which they transfer pollen, the efficiency with which they gather pollen within various crops, and the increased endurance to fly in adverse weather for longer periods of time (Stubbs and Drummond 2001, Stanghellini et al. 2002, Desjardins and Olivera 2006, Young et al. 2007). Further, the bumble bee has the ability to buzz pollinate or sonicate the flower for pollen, a pollination technique not seen in honey bees (Buchmann 1985, Desjardins and De Oliveira 2006, Goulson 2010, Heinrich 2004, King 1993, Kwon and Saeed 2003, Stubbs and Drummond 2001, Velthuis and van Doorn 2006). During buzz pollination, bumble bees attach their mandibles onto the anther of the bloom and vibrate their thoracic muscles thereby dislodging pollen from the flower. This is a very efficient method of gathering pollen from poricidal dehiscent crops such as tomatoes, eggplant, and cranberries (Buchmann 1985, Kwon and Saeed 2003, Stubbs and Drummond 2001). Other studies have also shown that bumble bee foraging activity starts earlier and ends later in the day than managed honey bees and they forage in lower temperatures (Desjardins and De Oliveira 2006,

Goulson 2010, Heinrich 2004). Honey bees begin to forage around 16 ºC or 60ºF, whereas bumble bees will start at 10ºC or 50ºF. Additionally their long tongues enable them to better pollinate flowers with deeper corollas than honeybees (Goulson 2010, Heinrich 2004, Velthuis and van Doorn 2006).

Although not often delineated from honey bees in the public eye, bumble bees differ greatly from their more popular counterpart. Foregoing details on the more obvious visual differences of the two genera (especially in relation to body size, color and abundance of hair) their nesting habits vary greatly as well. Native to many parts of the world, including North America, bumble bees have annual nests and typically nest underground. Bumble bee species are split loosely into two groups; pocket makers or pollen storers, depending on how they nest. As the weather begins to warm, most queens whom have recently emerged from solitary hibernation find suitable nest locations and begin the colony alone. The first eggs are laid in a 'brood clump' on top of pollen. In the first few weeks of nest development, the queen perches on the clump awaiting the emergence of the first of the brood that will eventually emerge and spin their own silken cocoons. In pocket makers, subsequent eggs will be laid in wax packets around the original cocoons, living and feeding off of pockets of pollen provided by the workers. In pollen storers, individual silken cells are spun for each larvae and it is here that they spend the majority of their development. Nectar and pollen are fed directly to the larvae by adult worker bees. This method of feeding allows for worker bees to control the size of the bee that will develop. As the job of each bumble bee is loosely determined by size, pollen storers may be able to control

the workforce ability of the next generation. Once development ceases, the old cocoons are cleaned and in many species are used to store pollen and honey (Heinrich 2004, Goulson 2010). Pollen storers are the only bumble bees that can currently be reared commercially. Pollen storers take pollen out of storage pots and feed individual young, directly, from those provisions. The use of storage pots makes nurse bees more likely to accept pollen provided by human rearers at any location within the nest. In pocket maker species, nurse bees will only provide pollen to a group of larva, and do not readily accept pollen provided by breeders. Much more care is needed to rear these species and therefore, are not available commercially (Goulson 2010, Velthuis and vanDoorn, 2004). In both types of bumble bees, fully developed brood will take over foraging and nest caring duties full time (Goulson 2010, Heinrich 2004, Vogt 1986).

Bumble bees generally incorporate plant fibers into their nest for insulation. This insulation blankets the nest in order to maintain an optimal temperature of 30ºC in the cooler months. Similarly, bumble bees are also known to cool their colony in the summer months by a fanning behavior. By rapidly beating their wings, bumble bees can cool off their nest in the middle of the season to maintain that same 30ºC (Goulson 2010, Heinrich 2004, Vogt 1986).

1.1.3 Commercial bumble bees

In the early 1980s, commercial rearing of bumble bees for pollination services began in The Netherlands. Within three years 95% of tomato growers in the country were using bumble bees in their greenhouses. By the 1990s commercial bumble bee

production made its way to the United States, with the new world species, *B. impatiens* and *B. occidentalis*. Due to drastic declines in native *B. occidentalis* populations, the species is no longer used (Goulson 2010).

Currently, there are two species of bumble bees commercially available globally, *B. terrestris* and *B.impatiens. B. terrestris* is native to Europe and *B. impatiens* is endemic to the eastern United States. In the United States, there are two companies that distribute commercial nests of *B. impatiens*, Koppert Biological Supply Company and Biobest®. Despite slight differences in commercial nest design, the general concept remains. Bumble bee colonies are reared and placed within plastic boxes, equipped with enough pollen and a sugar substance to satiate bees whilst shipping. A large cotton swab is also placed within the plastic box to allow bees to insulate their colonies. Two holes are engineered into each plastic box that allows the grower to control the activity of his/her bumble bee nest. These entrance holes are controlled by a plastic flap that either opens or closes all or one entrance hole. Two open holes indicate the free movement of bees into and out of the colony. Pushing the flap down provides for one open hole and allows only bees to enter as a valve blocks the access of bees to return outside. This ability for growers to control bumble bee activity can limit exposure to pesticide applications during crop bloom. Each plastic box is placed within a slightly larger cardboard box. Two, three or four cardboard boxes are then placed within a corrugated plastic box that protects these units from the elements. With the exception of 3 single strawberry colonies in 2011, this study

predominately used quads (four colonies within one box), but the unit of study during each analysis was at the colony level (see Figure 1.1).

Figure 1.1. Pictures of the different components of a commercial bumble bee quad. Top left, inside the individual colony unit. Top right, the whole individual colony unit. Bottom left, inside the quad with three out of the four colony units. Bottom right, the full quad. Photo credit, J. Marchese

Commercial bumble bees are now the leading avenue to pollinate greenhouse tomatoes, worldwide. Countries in which bumble bees are prohibited (due to concern of the establishment of an alien bumble bee species) are unable to compete in the greenhouse tomato market (Goulson 2010, Stanghellini et al. 1998). While the necessity of commercial bumble bees has been realized and well-studied in the greenhouse tomato system, little is known about the ability of bumble bees to perform in the open field.

1.1.4 Significance and rationale

Because of the aforementioned behavioral qualities of bumble bees and the availability of commercial bumble bee pollination units, grower's interests are on the rise in utilizing this native pollinator and many growers are starting to use bumble bee units in their fields. However, questions and problems are starting to surface about how to keep these pollination units active through the whole bloom period.

Koppert Biological Systems is one of the main suppliers of pollination units in the United States. Their web page (www.koppert.com) provides guidelines and usage tips for different fruit and vegetable crops. The colony life expectancy for these units depends on the crop and ranges from 6-12 weeks, but many growers in Delaware are reporting that colony growth and activity among the units is highly variable and often times short lived.

To-date there has been no research conducted in Delaware (and in most other states) that addresses how to best use these commercially produced bumble bee colonies in various crops and under differing field conditions. Consequently, there are few crop or site specific management strategies available for growers to enhance the performance and extend the longevity of these units (Desjardins and Oliveira 2006).

In order for such an investigation to occur, other details of the species biology must be explored. As bumble bee nests differ fundamentally from those of the honey bee, an ideal housing strategy must be developed that will optimize colony growth and productivity.

This project measured colony growth over the season in different crops, examined placement effects, and assessed overall bumble bee health and survival. We correlated bumble bee productivity measures with horticultural effects of yield and quality of the harvested crop. Studying these factors helped determine how to best use these pollinators and increase crop yields for growers. Specifically, we developed best management practices for commercial bumble bee units in strawberries, watermelon, and pickling cucumbers in the state of Delaware. These crop specific, best management practices will provide growers with guidelines on the most efficacious use of bumble bee units for their area. Ultimately, the goal was to produce recommendations to commercial growers in Delaware on how to best use *B. impatiens* for the pollination of these crops during the growing season.

1.2 Methods

The field investigation occurred during the spring and summer of 2011 and 2012 on various farms throughout Kent and Sussex Counties in the state of Delaware. There were two main areas of investigation, the biology of the bumble bee and the crop product, each with various subsets.

1.2.1 The biology of the bumble bee

The bumble bee study included various assays aimed at determining the most ideal conditions for a bumble bee nest. Commercial bumble bee colonies and shaded structures, currently being tested within the company, were obtained from Koppert Biological Systems. Commercial bumble bees were housed in the standard bumble bee quads (except in the spring of 2011 where single colonies were used for some of

the strawberry crop, placed in high tunnels). Throughout each growing season, all quads were situated in a variety of locations and conditions within each field. Location treatments were edge and in field placements. Colony condition treatments were full sun exposure without shade structure, shade structure (A-frame, pentagon, reflective A-frame and reflective pentagon), natural shade, and buried quads. Pictures of quad treatments used in watermelon and pickling cucumber crops over the 2011 and 2012 field seasons are below in figures 1.2-1.5.

Figure 1.2. 2011 Watermelon colony placement treatments, starting from the top left, A-frame, buried, pentagon, natural shade, and sun. Photo credit: J Marchese

Figure 1.3. 2012 Watermelon colony placement treatments, from left to right, natural shade, shade structure and sun. Photo credit: J Marchese

Figure 1.4. 2011 Pickling cucumber colony placement treatments, starting from the top left, reflective A-frame, pentagon, sun, reflective pentagon, and natural shade. Photo credit: J Marchese

Figure 1.5. 2012 Pickling cucumber colony placement treatments, shade structure and sun. Photo credit: J Marchese

Each quad was assigned one treatment. Figure 1.6, below, is an example schematic of how bumble bee quads were organized in a watermelon field in 2012.

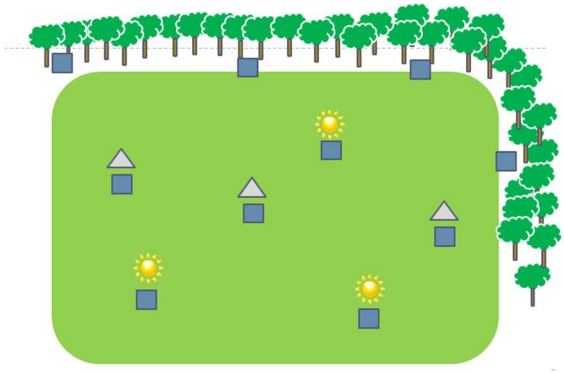

Figure 1.6. Generalized field schematic with quads assigned different location treatments. Each small box represents a quad (4 individual colonies) and its location treatment is denoted by a symbol in which the box is associated. A sun above the quad indicates the sun treatment, a triangle denotes shade structure, and the quads on the edge of the field by the trees were given a natural shade treatment.

Our goal was to test how the quad placement treatments affected colony productivity throughout the season. The main metrics used for productivity were colony weight, colony foraging and post mortem analyses that counted the number and type of cells and dead adult bees within the colony and the weight of the colony's wax.

In the watermelon and pickling cucumber fields, following the Goulson et al. (2002) study, each colony within each quad was weighed to the half gram on a weekly basis to determine productivity of the nest. During analysis, weight data was further scrutinized and several variables were extracted and compared against colony treatment and the field of the colony. These variables were mean weight throughout the season, the peak weight that each colony reached, the number of days it took for the colony to reach its peak weight, and the change in weight from the start weight at the beginning of the season, to the peak weight.

In 2011, colonies throughout the study were outfitted with a Bumble Bee Activity Recorder, provided by Dr. Peter Kevan of the University of Guelph in Ontario, Canada. These recorders were developed by Dr. Kevan's team for recording foraging activity of bumble bees in green houses, and to provide an accurate account of colony activity. The device determines how many bees enter and exit a colony in a given amount of time (Kevan et al. 2009). Unfortunately, these recorders did not work properly on our quads and data was not collected from these units. Colonies were also monitored weekly for foraging activity by counting foragers for 5 minutes at a time. All bees that entered and exited each colony were counted during a five minute period. In 2011, all foraging counts occurred in the morning hours between 7am and 11am. In 2012 only, a morning and an afternoon foraging count was conducted and the differences between the two were compared. Similar to weight data, data gathered from these foraging counts were analyzed to help researchers determine the foraging activity of the colonies throughout the season. In both years, foraging was further analyzed by extracting the following variables: total season long foraging, average foraging, peak or maximum foraging counted for each colony, and the day in which the peak foraging occurred. In 2012 all of these variables were analyzed separately between morning and afternoon counts and when applicable, combined total day counts were analyzed. All variables listed above were tested against either the placement treatment of each quad or the field in which the quad was placed. Additionally, data recorders (Hobo®) were used both years to measure temperature and relative humidity at half hour intervals throughout the study.

Each colony was analyzed at the end of the season or whenever it was found dead (no live bees remained within the single unit). The wax nest was carefully stripped from the cotton structure provided from Koppert Biological Systems and weighed. Each cell of wax was categorized by being either a queen cell (solely identified by its larger size) or non-queen cell. The number of cells in each category was recorded. Perished bumble bee specimens that remained in the colony were counted and categorized by caste. Other insect nest inquilines were identified to family and counted. In watermelon fields in 2012, the highest and lowest weighing colonies were sacrificed in the middle of the season and a post mortem analyses was conducted in the manner mentioned above. Researchers hoped that this sacrifice and post mortem analyses of these 6 colonies would help to relate weight to overall productivity.

After all post mortem analyses were complete, all wax and bumble bees collected were stored in -80^{0}C freezer and stored for later pesticide analysis. Wax and bees from colonies of fields in which a grower submitted season long spray recorders were combined and homogenized. Two separate 50 mL test tubes were filled with either bumble bees or wax from each field. Each vial designated a separate pesticide analysis in which the substrates were tested for a list of chemicals (3 fields of samples yielded 6 test tubes and 6 different analyses). Ultimately, this testing determined levels of chemical accumulation found in the bees and the wax in the colony. The season long pesticide records were compared to the results of the conducted analyses.

1.2.2 Experiment broken down by crop

In an attempt to understand crop yield, various measures were taken. Foragers with full pollen baskets were captured and their foraging relevance to the crop in which they were placed was analyzed by identifying pollen grains. Additionally, at the end of the season, fruit yield was determined by harvesting several transects throughout the field in cucumbers and watermelon. The location of each transect was based on the proximity to the colony and the location within the field. All experiments are explained in further detail, below.

1.2.2.1 Strawberry

In 2011 colonies were placed in closed strawberry high tunnels to accommodate an early harvest of the crop. When weather warmed, high tunnels were opened. This was recorded starting 4/27/11. Quads were then placed outside of the high tunnels for the later crop in the field. In 2012 colonies were placed strictly in an exposed field setting. Pollen was collected from workers of colonies in both treatments and pollen content was compared using a hemocytometer. Pollen pellets were diluted to a concentration of 1.4×10^{-4} µL/g with DI water and 5 µL of fuschin stain solution. Then, 20 µL of the pollen mixture was inserted into the hemocytometer and all pollen grains within the middle square were counted and identified.

Hobo® data recorders were placed within the inner cover of the colony cardboard box, resting on the top cover of the individual colony's plastic box. The number of Hobos® were limited and there was never more than one Hobo® per quad. Hobos ® were used to determine colony thermoregulation by measuring temperature

and relative humidity. Ultimately, the early nature of the strawberry season allowed for the greatest observation of these pollinators and their fecundity throughout the season.

1.2.2.2 Watermelon

Many aspects of the project mentioned above were demonstrated on watermelon plants. The focus of the watermelon study was the placement of the quad to increase productivity of the colonies and yield of the crop. There were several treatments of the quads to vary their exposure to sun. Several shade structures, developed by Koppert Biological Systems, were used as well as varied placements of the quads within the field and along the edge of the field in natural shade. The same 3 growers participated in the study for both years. Each field corresponds to one of the growers. Despite keeping the grower constant, only field 1 was the same field for both years. In 2012, growers 2 and 3 provided different fields for the experiment than was provided in the previous year. In 2011, 20 quads were allotted between 3 fields. Field 1 received 6 colonies, field 2 received 4 and field 3 had 10 colonies. The treatments were sun, natural shade, A-frame shade structure, pentagon shade structure, and buried. The buried treatment was removed in 2012 as these colonies were hard to maintain and did not fair the elements well. In 2012, all fields received 10 quads, each randomly assigned with one of the three placement treatments (sun, natural shade, shade structure (analogous to the 2011 A-frame). Therefore, each field received three quads of each treatment (3 quads x 3 treatments = 9 quads). The remaining quad also received one of the three treatments, and each field's 10^{th} quad had a different

treatment. The extra treatment was allotted in this way: field 1: natural shade, field 2: shade structure, field 3: sun.

Weekly colony weights, measured to the half gram, were collected throughout the season. Further, each week, foraging data were taken by counting bees entering and exiting the colony for 5 minutes. This experiment was conducted once weekly in 2011. In 2012, a morning and an afternoon foraging count was done separately to examine the differences of foraging with time of day. Relative humidity and temperature were measured in Hobo® data loggers placed in colonies throughout the field in the same location within the inner cardboard box, as mentioned above in the strawberry crop. Foraging activity data was gathered by counting the number of flower visits per minute/bumble bee.

In 2011 watermelon surveys were conducted by horticultural researchers located at the University of Delaware Elbert N. & Ann V. Carvel Research and Education Center. These surveys persisted throughout the season and the following metrics were taken on a weekly basis on each of 10 randomly selected plants per field (5 pollenizers and 5 seedless plants), fruit number, location of first fruit node, all fruit node locations, the number of fruit abortions, the node location of each fruit abortion, the vigor and health rating, the leaf and crown temperature, the number of and location of all flowers, the number stems and branches, the longest stem length, and the number of nodes of each of the longest stems (Johnson 2011). Data was collected from fields with both *Apis* and *Bombus* pollinators from both Delaware and Maryland. In 2012 the watermelon experiments were conducted by the researchers of this project.

Two separate experiments were run focusing on fruit set and harvest. To determine fruit set, two surveys were conducted in each watermelon field that determined fruit set number in a plant at five set locations at 10-50 meters away from each quad. The first survey was completed when it was determined that the fruit within the field reached roughly the size of a softball. The second was performed just before the first harvest. Due to confounding factors with third party harvesters, a full harvesting experiment was only conducted on one out of the three watermelon fields. For three harvests, a 30 foot transect was set immediately surrounding a quad (two quads per treatment) and harvestable fruit from plants within each transect were harvested and weighed.

1.2.2.3 Pickling Cucumber

Treatments of pickling cucumber colonies also varied between the two years. In 2011, colonies were either placed in the sun, natural shade or one of three different shade structures (reflective A-frame, reflective pentagon, or pentagon). In 2012, colonies had sun or shade structure (A-frame) only. Colonies designated for the pickling cucumber crop were purposely placed in multiple fields to test their ability to be successfully transported. In 2011, half of the quads were moved after harvest and in 2012 all quads were moved together from one field, to the next. In 2011, all 20 colonies placed in field 1 were then moved to the second field after harvest. Colonies existed in field 2 until the end of the season when they were announced 'dead' after all living bees had died or had left the colony. In 2012, all 10 quads were placed within the same field and then transferred to a second field after harvest. These quads were

then subsequently transferred to a pumpkin field after harvest of the second pickling cucumber field.

Experiments in the pickling cucumber field focused on which pollinators were actively in the field pollinating the crops. Pollinators (native or commercial) were then collected along 5 (in 2011) or 6 (in 2012), 50 meter transects for 30 minute intervals to determine what species were actively on the plants. The last foraging tests mirrored those conducted in the strawberry and watermelon crops. Bees entering and exiting the colony during a five minute period were counted. Each colony in each quad was weighed to the half gram, weekly. Performance differences in the experiments were compared to determine the hardiness of bees after being transferred. Crop yield data was gathered by harvesting eight, 20 foot transects on various areas of the field. Those areas were labeled as follows: field edge near placed bee colonies, field edge away from placed bee colonies, middle of field near placed bee colonies, and middle of field away from placed bee colonies. GPS data points of each transect were taken to mark distance from quad. Finally, in 2012 experiments were attempted that focused on the bumble bees' ability to transfer pollen from flower to flower. For these experiments flowers were bagged the day before opening and were randomly assigned a pollinator visiting number of 0, 1, 2, 4, or 7 following the 2011 study by Artz and Nault. This designation determined the number of bumble bees that were allowed to visit the flower before it is was bagged for further isolation. Researchers waited for bumble bees to land on the selected flowers. This experiment was not

completed as bumble bee foragers were not in the field in the numbers to make this experiment feasible.

1.2.3 Statistical analyses

Statistics were completed using SAS and through the aid of McDonald 2009. The one way anova, model I test was run on most data sets when comparing colony treatments (natural shade, various shade structures, buried, or sun) with the variable tested. Variables tested were derived from the weekly, five minute foraging counts, the weekly weight, and data gathered during colony post mortem analyses. Each variable that was tested against colony treatment was also tested again against the different fields in which the colonies were placed. Further, all analyses separated colonies in different crops. For data sets that did not meet the homoscedastic assumption, a Welch's anova was run, when appropriate. When statistical significance was detected, a Tukey Kramer or Dunnett's T3 post hoc tests were used for the one way anova or Welch's anova, respectively. Further, many of the experiments comparing data from two data sets were analyzed using linear regression/correlation. In the case of data sets not meeting the normal distribution and homoscedastic assumptions the Spearman's rank test was used as a substitute.

1.3 Results

1.3.1 Strawberry

1.3.1.1 Temperature and relative humidity

Temperature (Figure 1.7) and relative humidity (Figure 1.8) were higher on average throughout the season in the high tunnels in 2011 than they were in colonies placed in the field, (temperature: $F_{1, 10748}$=35.19, P<0.0001, RH: $F_{1,10748}$=53.36, P<0.0001).

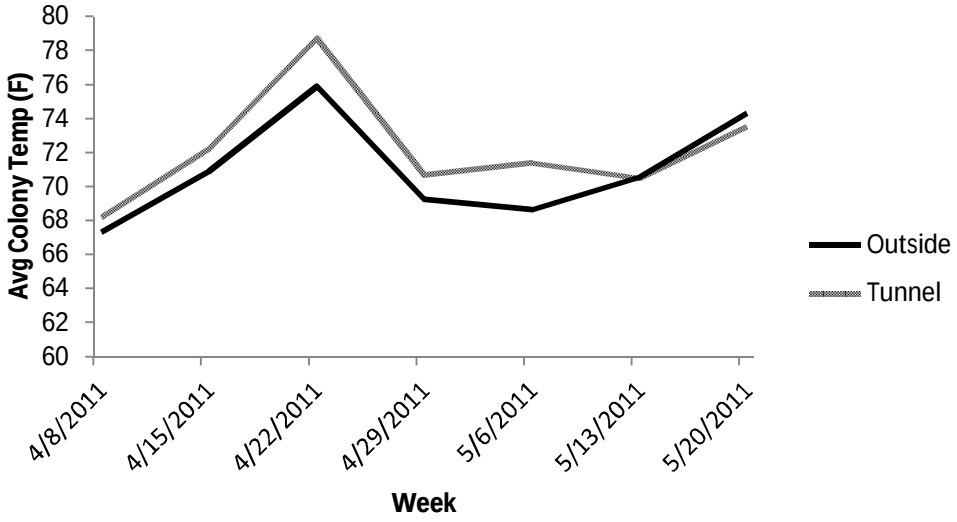

Figure 1.7. 2011 Strawberry, average weekly temperature by placement of colony.

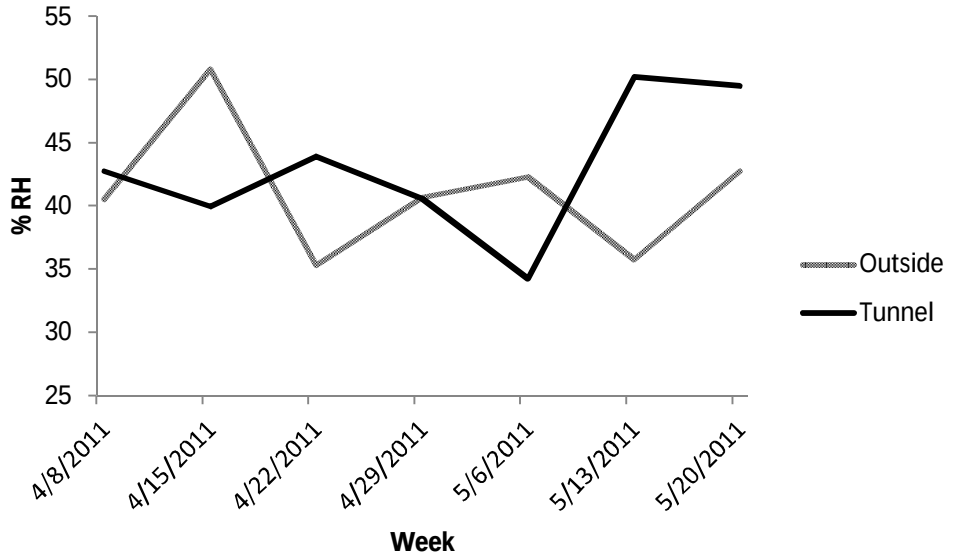

Figure 1.8. 2011 Strawberry, average weekly percent relative humidity by placement of colony.

1.3.1.2 Pollen

In 2011 strawberry pollen was the most common strawberry grain seen in the pollen samples within the strawberry crops. It was present in 45% of samples. The other types of pollen most commonly seen were peach (18%), Japan Quince (18%), and English Plantain (18%). In 2012 out of the 24 strawberry samples taken from the corbiculas of the commercial bees within the field, 2 samples contained strawberry pollen. The most common pollen grains that were found in the strawberry samples and the percentage of samples that they were found in were peach (33%), eastern redbud (25%), sedum (20.8%), blueberry (16.67%), and mullein (16.67%).

1.3.2 Watermelon

1.3.2.1 Five minute foraging counts

In comparing treatments by total foraging of a colony through the season all colonies placed with shaded treatment yielded higher total foraging counts than those placed in the sun (Figures 1.9, 1.10),(2011: $F_{4, 71}$=9.93, P<0.0001, 2012: $F_{2, 99}$=8.31, P=0.0005).

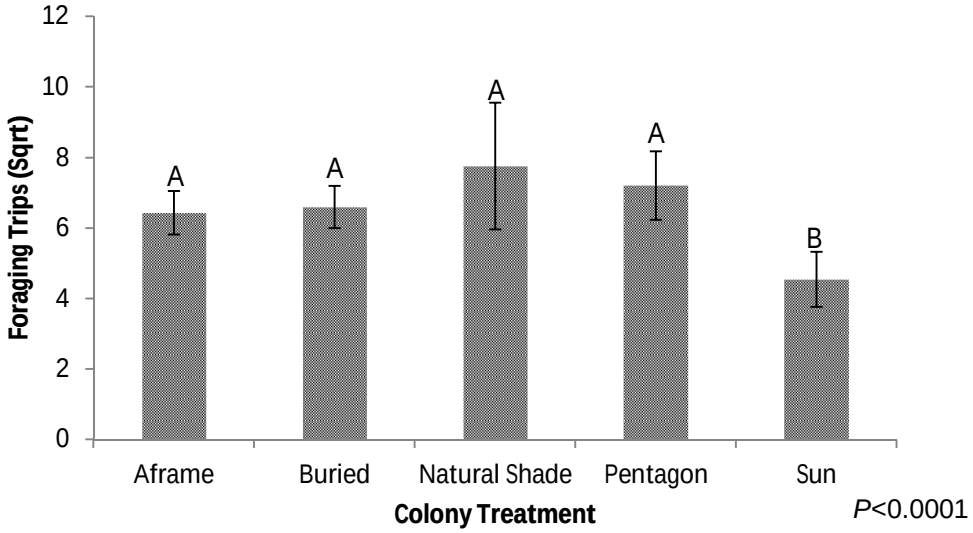

Figure 1.9. 2011 Watermelon, total season foraging by colony treatment (square root transformed). Error bars indicate 95% confidence intervals, and letters denote statistical significant differences as determined by Tukey Kramer post hoc test.

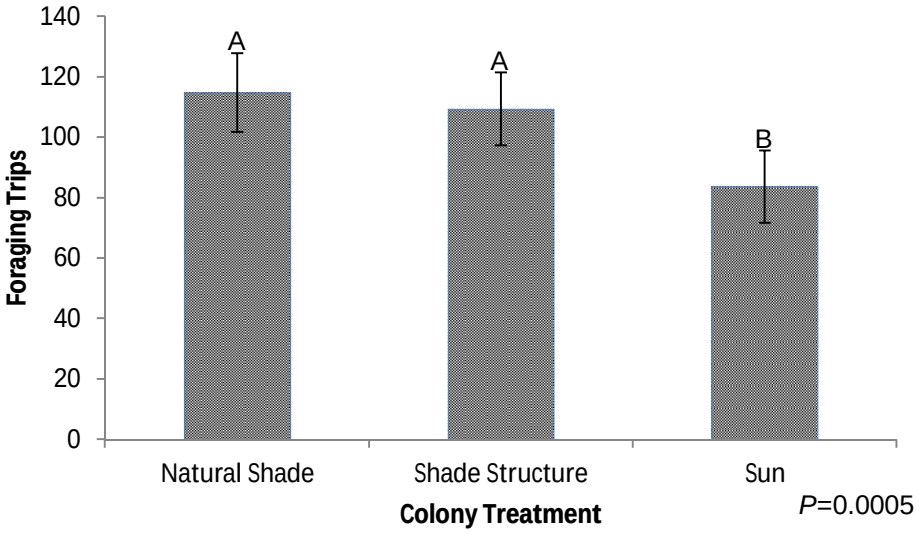

Figure 1.10. 2012 Watermelon, total season foraging by colony treatment. Error bars express 95% confidence intervals, letters denote statistical significant differences determined from Tukey Kramer post hoc test.

Total forage of colonies by field was statistically significant in both years. In 2011, colonies in field 1 foraged more, on average, than those in field 2 (Figure 1.11). In 2012, both fields 1 and 3 foraged more, on average, than field 2 (Figure1.12), (2011: $F_{2, 46.24}$=6.38, P=0.0036, 2012: $F_{2, 99}$=6.08, P=0.0032).

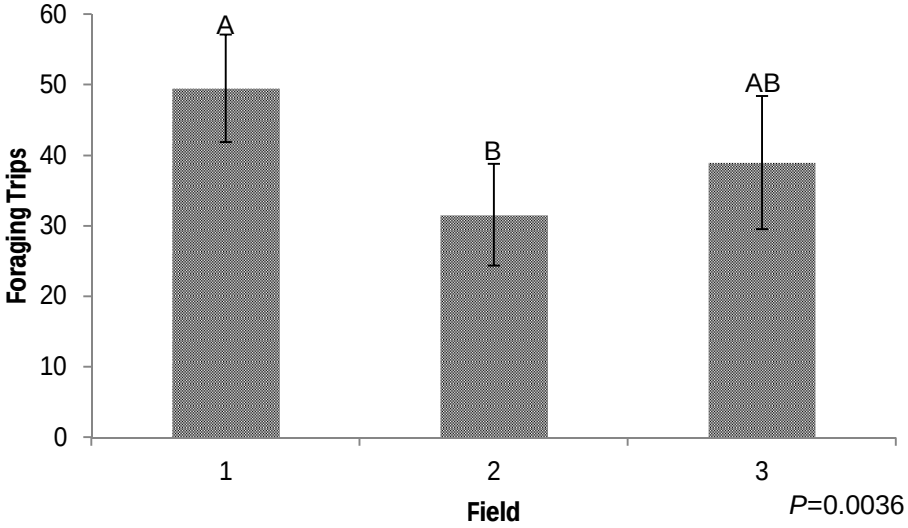

Figure 1.11. 2011 Watermelon, total season forage by field. Error bars denote 95% confidence intervals and letter designate statistically significant differences as determined by Dunnett's T3 post hoc test.

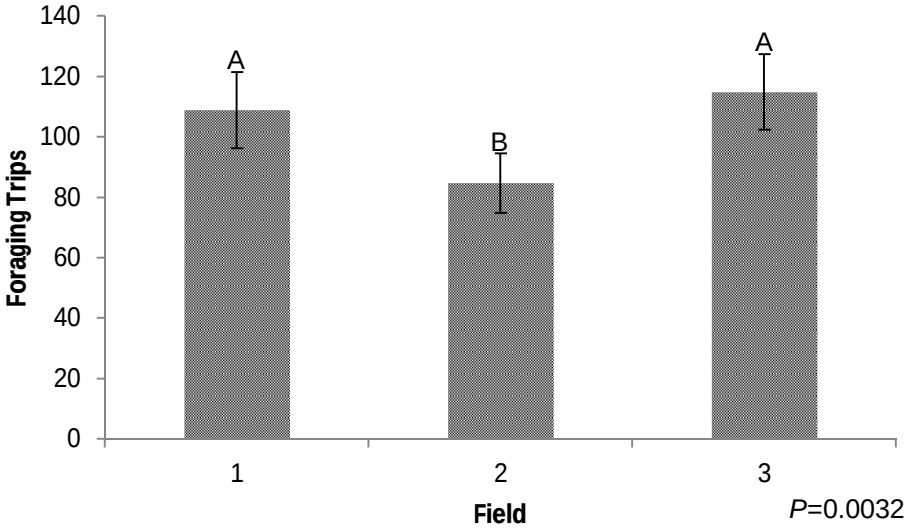

Figure1.12. 2012 Watermelon, total season foraging by field. Error bars denote 95% confidence intervals and letters designate significant differences by Tukey Kramer post hoc test.

The season long, average forage of a colony was tested against the treatment in which the colonies were placed. Statistically significant differences were only detected in the 2011 analysis. During that year, all non-sun treatments, on average, had higher total season forage averages than those colonies placed in the sun (2011: $F_{4, 24.87}$=6.79, P=0.0008, 2012: $F_{2, 99}$=2.8, P=0.0654). Mean foraging of colony by field was significant in 2011 and 2012. In both years, field 1 had higher forage averages than field 3 (2011: $F_{2, 45.5}$=5.09, P=0.0101, 2012: $F_{2, 99}$=4.51, P=0.0134). Each colony's peak forage counts and the date of the peak was extrapolated and compared by treatment and field. In 2011, the A-frame shade structure and the sun treatments had lower peak foraging numbers, on average, than the other colony treatments (Figure 1.13). In 2012, no difference between treatments was detected (Figure 1.14), (2011: $F_{4, 24.62}$=4.76, P=0.0055, 2012: $F_{2, 98}$=1.22, P=0.2986).

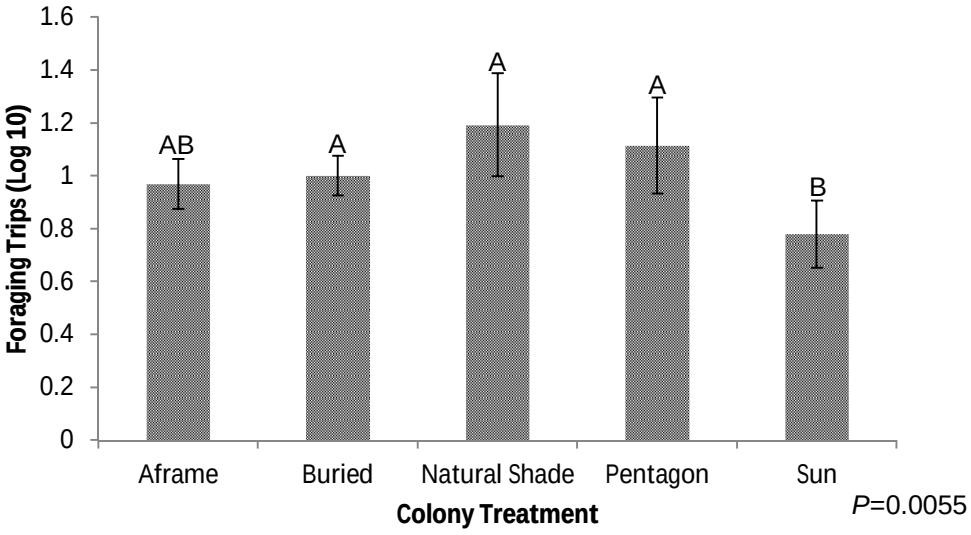

Figure 1.13. 2011 Watermelon, peak forage counts by colony treatment (log10 transformed). Error bars denote 95% confidence intervals and letters demonstrate statistically significant differences as determined by Dunnett's T3 post hoc test.

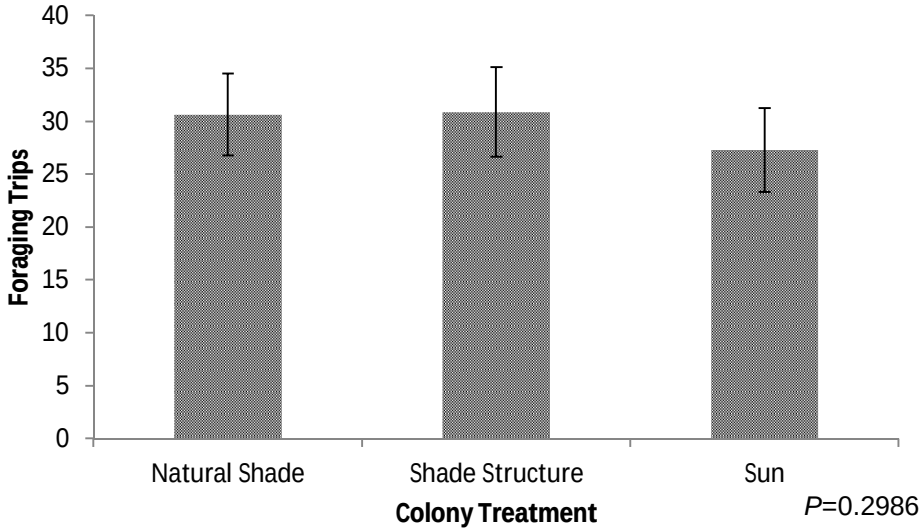

Figure 1.14. 2012 Watermelon, peak forage counts by colony treatment. Error bars denote 95% confidence intervals.

The different fields differed in their peak foraging counts in both years. Field 1 had higher peak forage counts than the other two fields in 2011 (Figure 1.15), whereas in 2012, field 1 had higher peak foraging counts than field 2 (Figure 1.16), only, (2011: $F_{2,45}$=6.67, P=0.0029, 2012: $F_{2,99}$=4.07, P=0.0201).

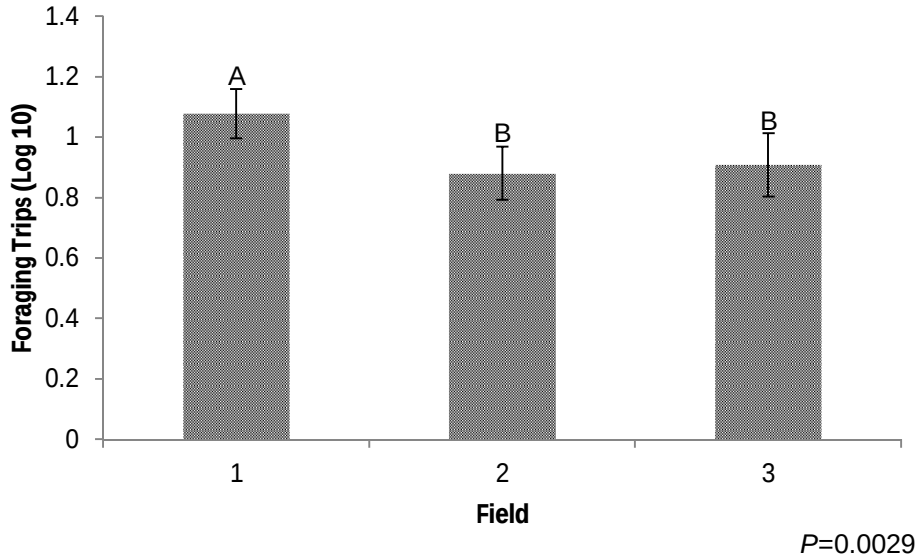

Figure 1.15. 2012 Watermelon, peak forage counts by field (log10 transformed). Error bars demonstrate 95% confidence intervals and letters signify statistically significant differences between fields, determined by a Dunnett's T3 post hoc test.

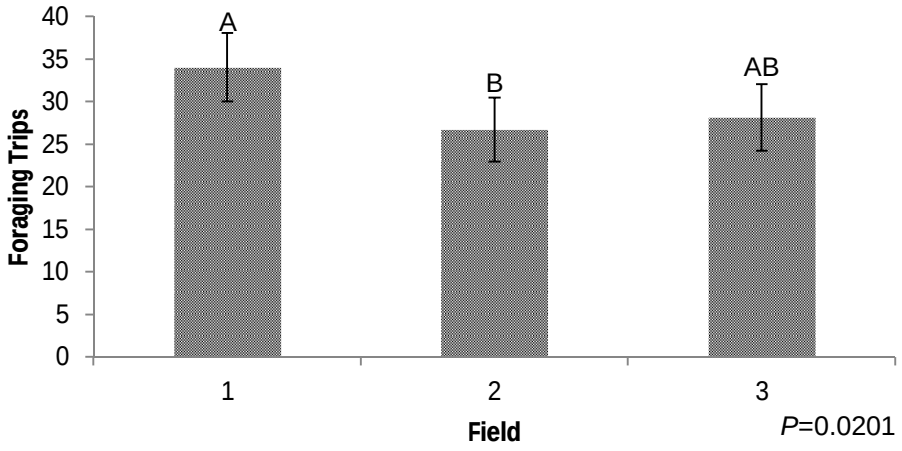

Figure 1.16. 2012 Watermelon, peak forage counts by field. 95% confidence intervals are demonstrated by error bars and letters designate significant differences, determined by a Tukey Kramer post hoc test.

The day in which an individual colony performed their peak forage was calculated to indicate the length of time the colony was still actively building up before decline in activity began. When compared by treatment, days to peak forage was statistically significant in 2011 (Figure 1.17), but not in 2012 (Figure 1.18). In 2011 the natural shade treatment took more days to reach peak than both the sun treatment and the buried treatment, (2011: $F_{4, 71}$=4.72, P=0.0019, 2012: $F_{2, 98}$=1.39, P=0.2532).

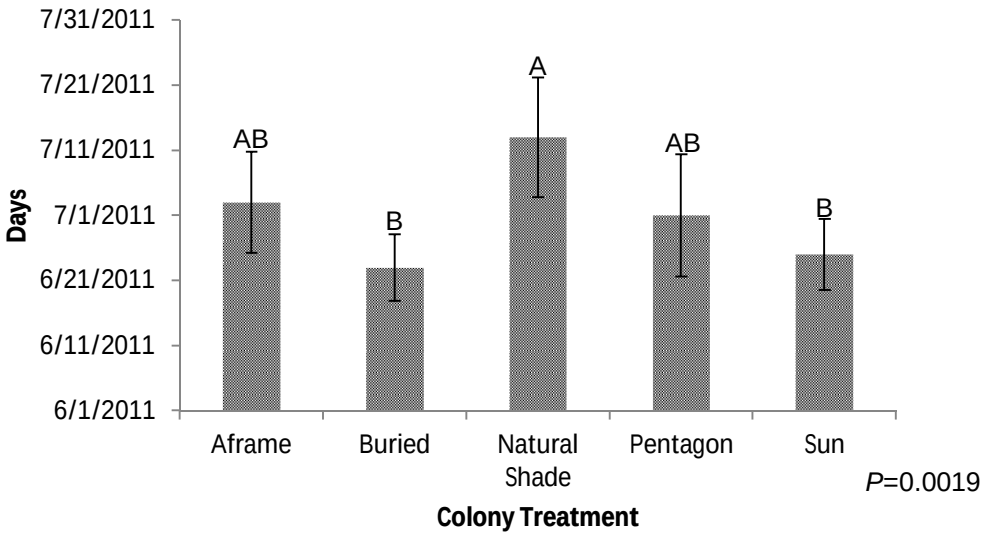

Figure 1.17. 2011 Watermelon, days to peak forage by colony treatment. June 1, 2011 represents the day in which the bumble bee quads were placed within the field. Error bars denote 95% confidence intervals and letters denote significant differences, determined by Tukey Kramer post hoc test.

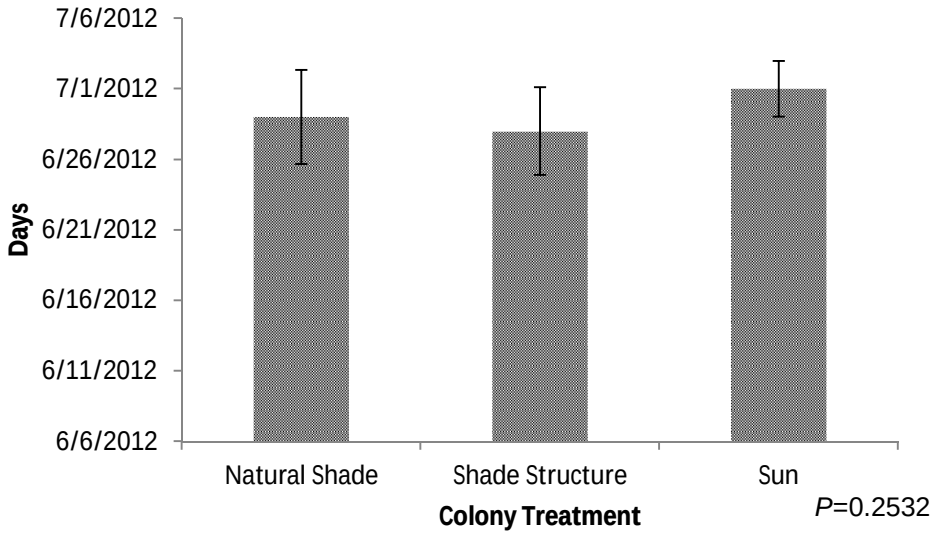

Figure 1.18. 2012 Watermelon, days to peak forage by colony treatment. June 6, 2012 represents the day in which the bumble bee quads were placed within their respective watermelon fields. No statistical significance was detected between treatments.

The day that a colony reached its peak forage was analyzed by the field in which it was placed. Again, in 2012, colonies in field 2 declined more quickly (less days to peak forage) compared to the other two fields (Figures 1.19, 1.20), (2011: $F_{2, 73}$=2.61, P=0.0802, 2012: $F_{2, 99}$=25.10, P<0.0001).

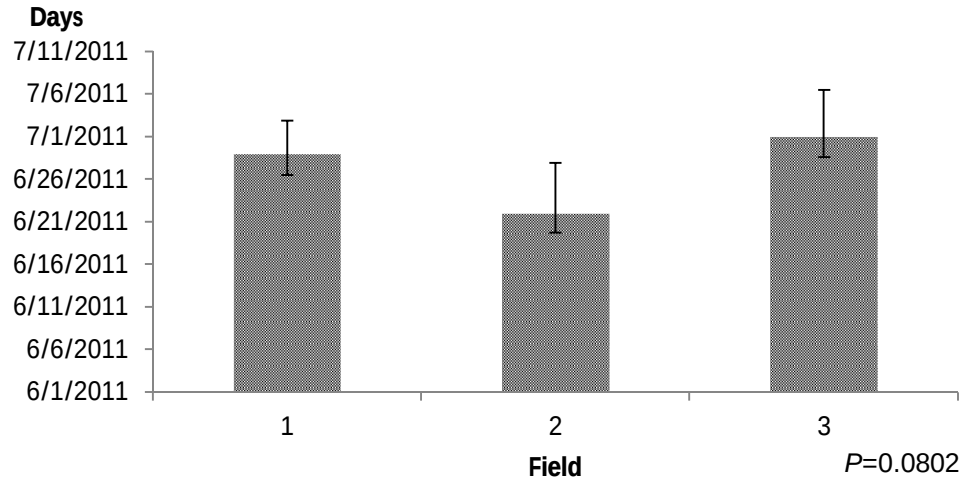

Figure 1.19. 2011 Watermelon, days to peak forage by field. Error bars denote 95% confidence intervals. June 1, 2011 represents the day in which the colonies were placed in the field. No statistical significance was detected.

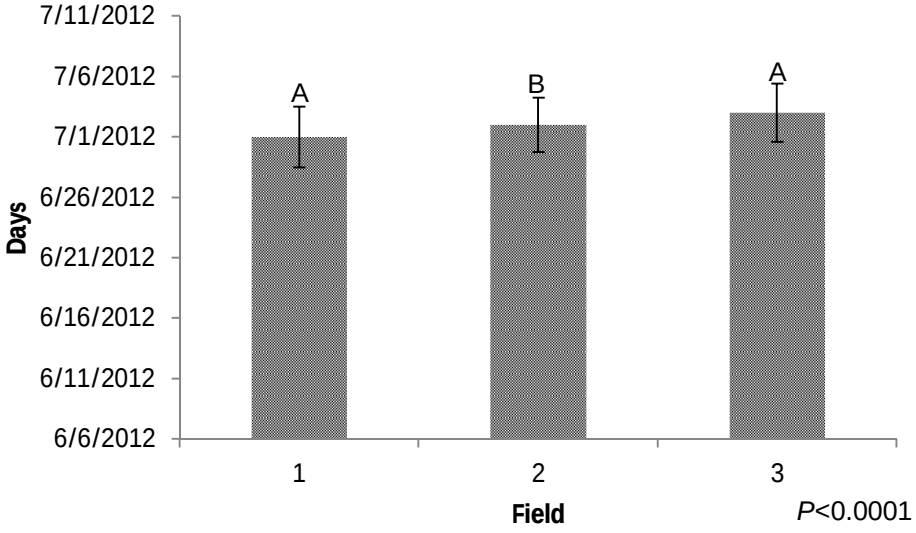

Figure 1.20. 2012 Watermelon, days to peak forage by field. Error bars denote 95% confidence intervals and letters denote statistical significant differences as determined by Tukey Kramer post hoc test. June 6, 2012 represents the day in which the colonies were placed in the field.

In 2012, two foraging counts were conducted on the same day each week that aimed to reflect morning and afternoon foraging. Treatments did not differ for peak morning foraging ($F_{2, 99}$=2.15, P=0.1221), whereas differences between fields were detected where field 2 had a lower morning peak than field 1 ($F_{2, 99}$=4.55, P=0.0129). In the afternoon differences were detected by treatment, where the shade structure was the only treatment with higher peak forage counts than the sun treatment, but no difference was detected between the different fields (treatment: $F_{2, 99}$=4.45, P=0.0141, field: $F_{2, 99}$=3.02, P=0.0534). The days in which the colonies peaked for their morning counts were not different when compared to their treatment but differed when compared to their field in which they were placed. Field 2 started declining in fewer days than the other two fields (treatment: $F_{2, 99}$=1.77, P=0.1757, field: $F_{2, 99}$=16.11, P<0.0001). The number of days that the afternoon foraging counts reached their peaks varied by site but not by treatment, field 2 reached its peak earlier than the other two fields (treatment: $F_{2, 99}$ =2.23, P=0.1134, field: $F_{2, 99}$=7.17, P=0.0012). We found that colonies generally foraged significantly more in the morning hours (before 11am) than they did in the afternoon (after 11am). Total season counts of morning foraging were measured against total season afternoon foraging counts, by treatment. Difference was seen by treatment, by time of day and by their interaction, treatment x time of day foraging activity of the colony was higher in the morning hours than in the afternoon, but the quad treatment affected the level of the differences seen (Figure 1.21), (treatment: $F_{5, 198}$=4.43, P=0.0131, time: $F_{5, 198}$=8.54, P=0.0039, treatment x time: $F_{5, 198}$=4.29, P=0.0149).

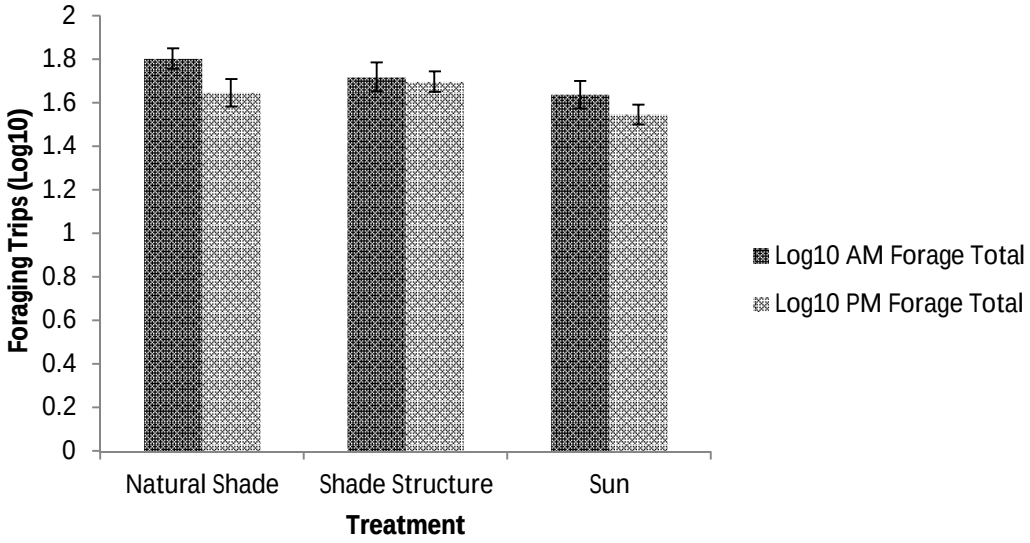

Figure 1.21. 2012 Watermelon, total morning and afternoon forage counts by colony treatment (log10 transformed). +/- 95% confidence intervals.

The same test was run by site where the total recorded foraging during the morning hours were compared to the total recorded foraging during the afternoon hours. Site and time of day came out with differences, the interaction term, site x time, did not, where total morning foraging was higher than total afternoon foraging and field 3 had the highest total foraging (Figure 1.22), (site: $F_{5, 198}$=6.05, P=0.0028, time: $F_{5, 198}$=8.45, P=0.0041, site x time: $F_{5, 198}$=1.46, P=2.344).

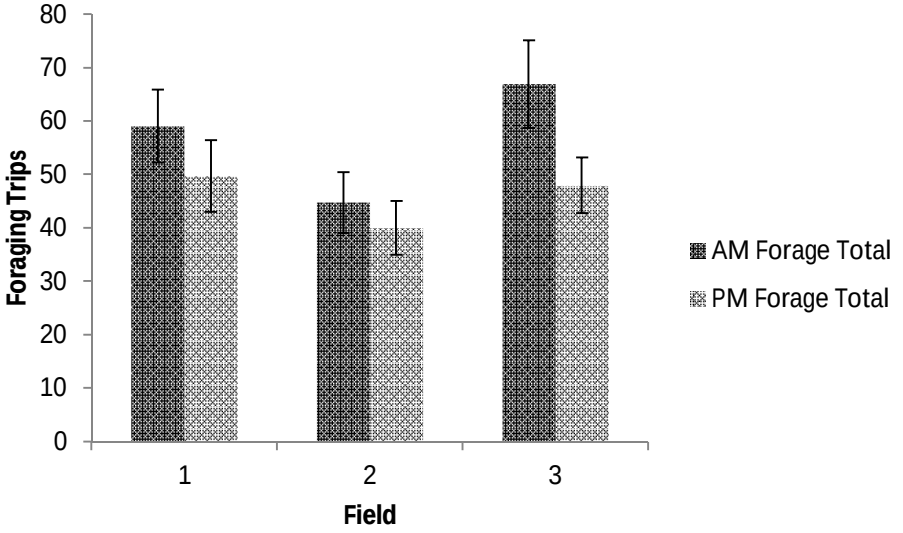

Figure 1.22. 2012 Watermelon, total morning and afternoon forage counts by field. +/- 95% confidence intervals.

1.3.2.2 Weight

Each colony was weighed to the half gram, weekly, throughout the season. Once there were no longer any living bees within the colony, it was considered 'dead' and a final death weight was taken. Average weight of each colony was taken and compared to its treatment. In 2011, all non-sun treatments had, on average, heavier colonies than those placed in the sun. In 2012 natural shade and shade structure treatments had higher average weights than those in the sun (2011: $F_{4, 25.62}$=11.67, P<0.0001, 2012: $F_{2, 99}$=8.29, P=0.0005). Average weight was also compared among the different fields in which the colonies were placed. In 2011, field 3 had lower weights than the other two fields, (2011: $F_{2, 45.48}$=5.40, P=0.0079, 2012: $F_{2, 99}$=0.074, P=0.4784). Change in weight from the beginning of the season to the peak weight showed how much a colony gained at the height of the season. In 2011, the means of

all shaded and the buried treatments were higher than the sun treatments (Figure 1.23). In 2012 the natural shade had the highest weight change, followed by shade structure treatments, then the sun (Figure 1.24), (2011: $F_{4, 75}$=29.87, P<0.0001, 2012: $F_{2, 99}$=21.07, P<0.0001).

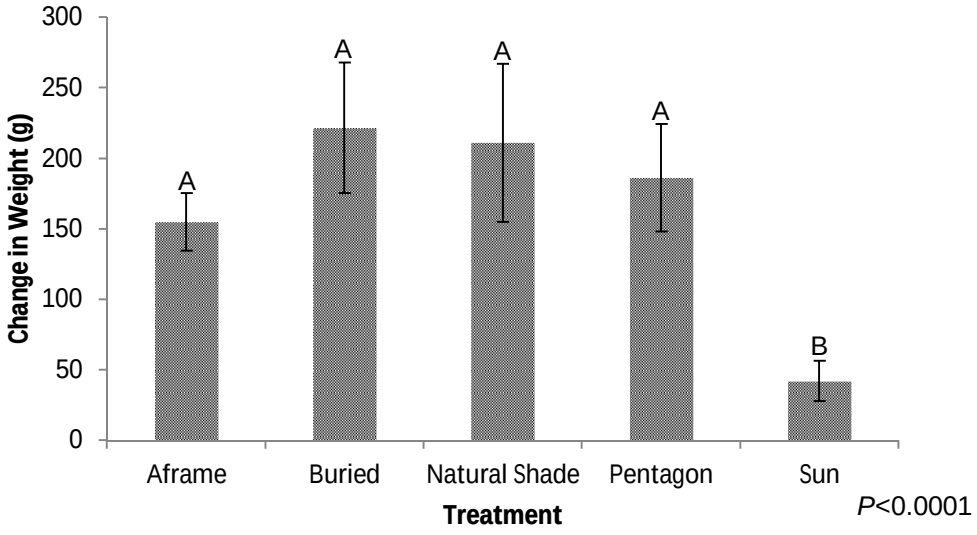

Figure 1.23. 2011Watermelon, colony weight change from the start to peak weight by colony treatment +/- 95% confidence intervals. Letters denote statistical differences as determined by Tukey Kramer post hoc test.

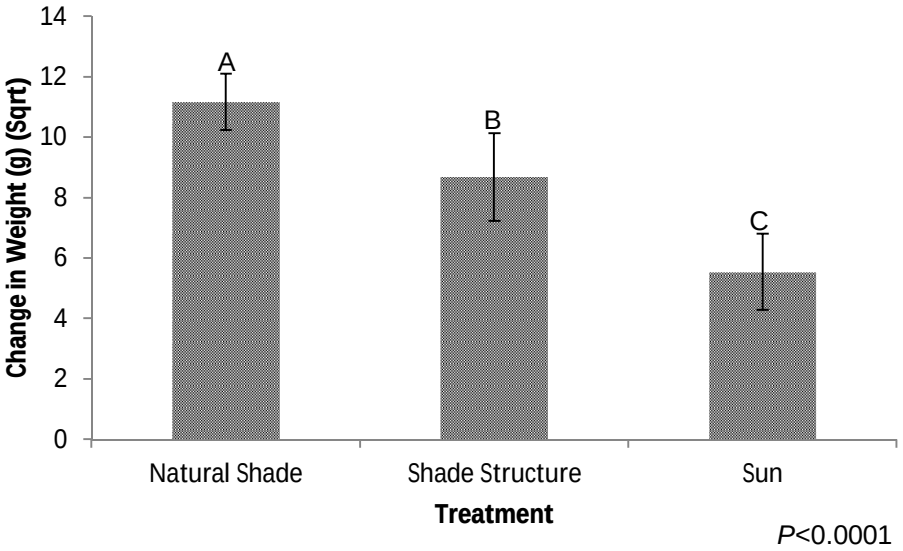

Figure 1.24. 2012 Watermelon, colony weight change from the start to peak weight of by colony treatment (square root transformed) +/- 95% confidence intervals. Letters denote statistical differences as determined by Tukey Kramer post hoc test.

Change in weight from the start of the season to the peak by field did not differ in either year (Figures 1.25- 1.26), (2011: $F_{2, 77}$=2.32, P=0.1052, 2012: $F_{2, 99}$=2.93, P=0.0578).

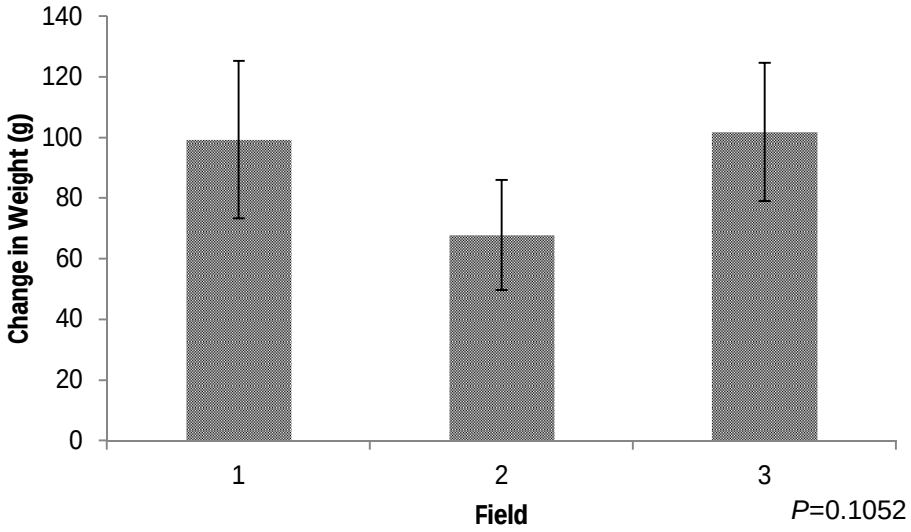

Figure 1.25. 2011 Watermelon, colony weight change from the start to peak weight by field +/- 95% confidence intervals.

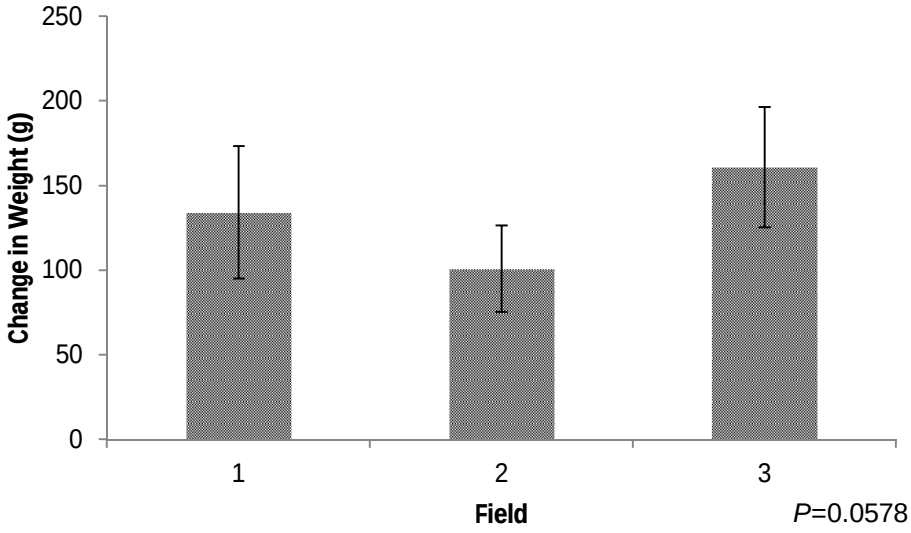

Figure 1.26. 2012 Watermelon, colony weight change from start to peak weight by field +/- 95% confidence intervals.

Peak weight of each colony by treatment was statistically significant in both years. In 2011, all non-sun treatments had, on average, heavier colonies at their peaks

than the sun colonies. In 2012, both shaded treatments had higher peak weights than the sun treatment (2011: $F_{4, 24.68}$=18.39, P<0.0001, 2012: $F_{2, 99}$=10.53, P<0.0001). Peak weights did not differ by field with a Tukey Kramer post hoc. (2011: $F_{2, 77}$=2.09, P=0.1312, 2012: $F_{2, 99}$=3.2, P=0.0451). The number of days it took for the colony to reach its peak weight was tested against its treatment and field. In both years all shaded treatments took a longer time to reach their peak weight than those colonies in the sun (Figures 1.27, 1.28), (2011: $F_{4, 75}$=17.69, P<0.0001, 2012: $F_{2, 93}$=16.59, P<0.0001).

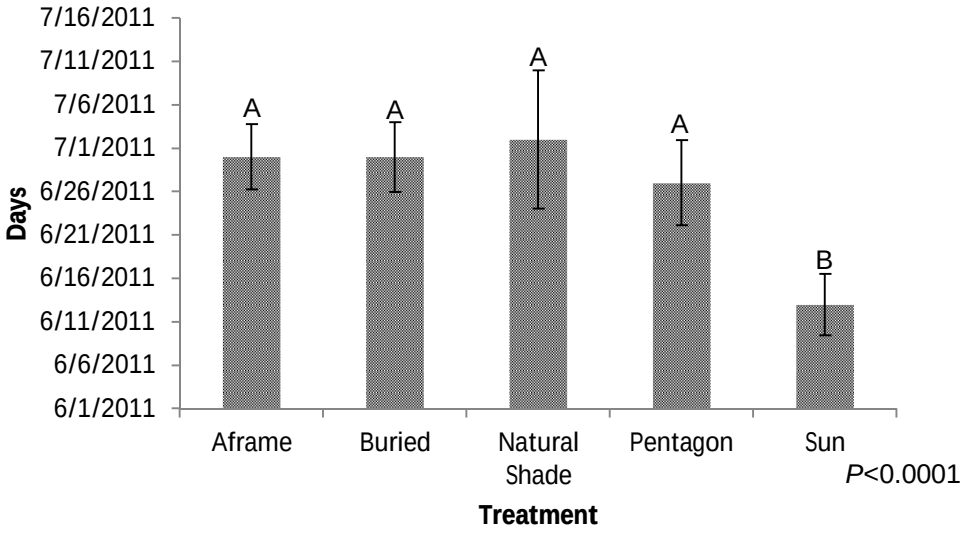

Figure 1.27. 2011 Watermelon, days to peak weight by colony treatment, +/- 95% confidence intervals. Letters designate statistical differences by a Tukey Kramer post hoc test.

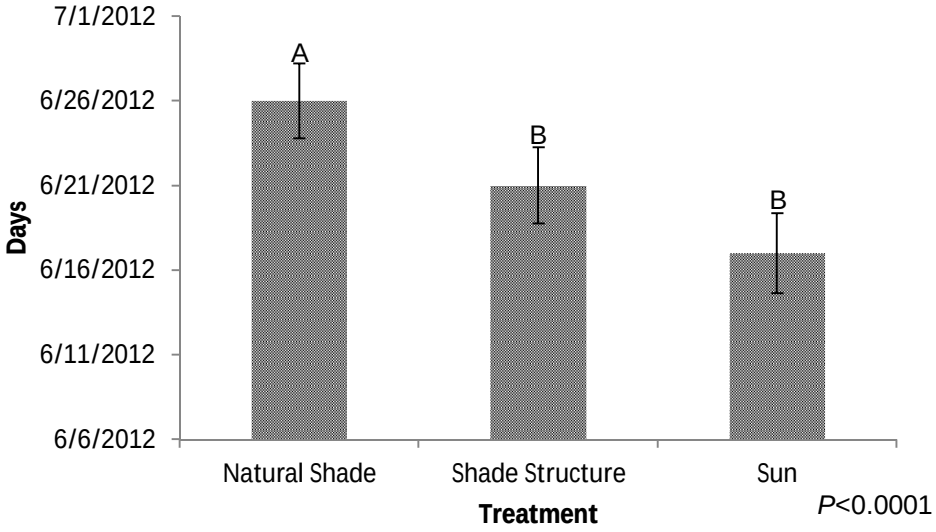

Figure 1.28. 2012 Watermelon, days to peak weight by colony treatment +/- 95% confidence intervals. Letters designate statistical differences by a Tukey Kramer post hoc test.

By field, peak weight date did not differ in 2011(Figure 1.29) but in 2012 (Figure 1.30), colonies in field 3 took a longer time to reach peak weight than the other two fields, (2011: $F_{2, 77}$=1.0, P=0.3727, 2012: $F_{2, 99}$=6.98, P=0.0015).

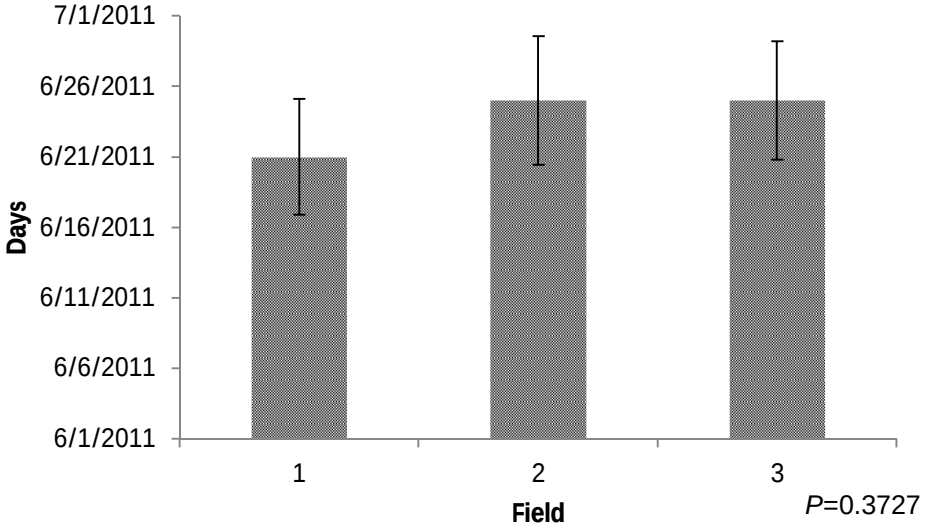

Figure 1.29. 2011 Watermelon, days to peak weight by field +/- 95% confidence intervals.

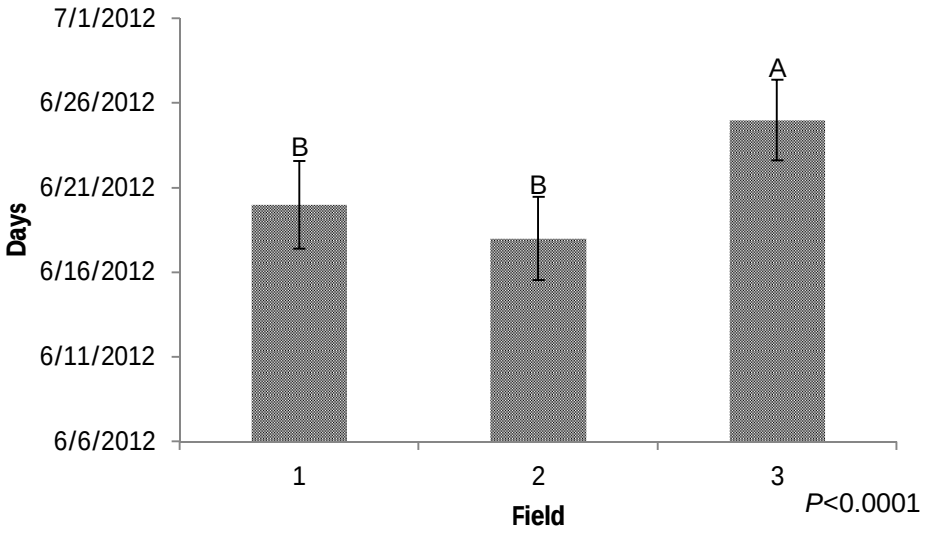

Figure 1.30. 2012 Watermelon, days to peak weight by field, +/- 95% confidence intervals. Letters designate statistical differences by a Tukey Kramer post hoc test.

1.3.2.3 Colony post mortem analysis

At the end of the season when each colony was determined to be 'dead', the colony was collected and analyzed. Due to an increase in availability of freezer storage in 2012 (to preserve colonies from decomposition while waiting to be analyzed), much of the data available is from 2012, unless otherwise stated. The number of total cells counted in each colony was compared against the treatment that it was given and the field in which it was placed (Figure 1.31). Both shaded treatments had more total cells than sun treatments, no difference was detected between fields (Figure 1.32), (treatment: $F_{2, 91}$=12.08, P<0.0001, field: $F_{2, 55.53}$=0.14, P=0.8731).

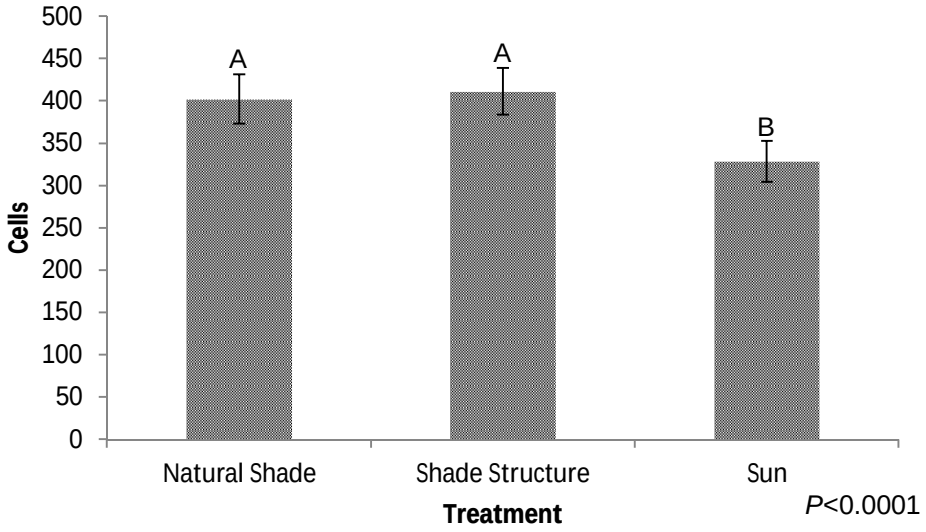

Figure1.31. 2012 Watermelon, total cells by colony treatment. Error bars denote 95% confidence intervals, letters denote significance difference by a Tukey Kramer post hoc test.

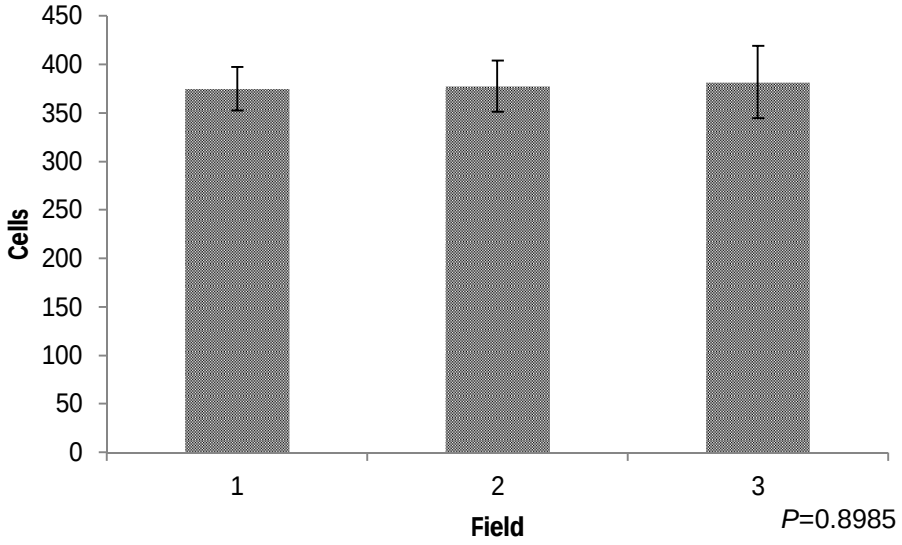

Figure 1.32. 2012 Watermelon, total cells by field. Error bars designated 95% confidence intervals.

Cells were further broken into queen vs non queen cells and both were tested against the colony treatment and field. All shaded treatments had more non queen

cells than the sun treatment ($F_{2, 91}$=9.82, P=0.0001), the same analysis by field had no differences ($F_{2, 91}$=0.23, P=0.7965). No differences were detected between treatments of fields and the number of queen cells (treatment: $F_{2, 91}$=1.75, P=0.1789, field: $F_{2, 92}$=1.36, P=0.2611). Peak colony weight was compared to the total number of cells with correlation/linear regression (Figure 1.33), (r^2=0.2907, df=92, P<0.0001).

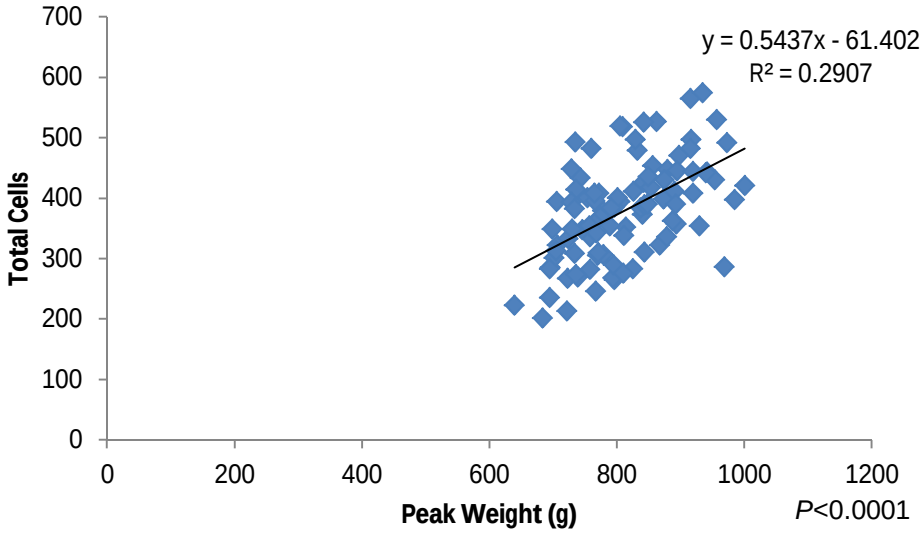

Figure 1.33. 2012 Watermelon, total cells versus peak weight.

The reported end weight of the colony vs total cells was compared (ρ=0.4589, df=92, P<0.0001). Average weight of the colony and total cells were also compared to the total cells of the post mortem count, (ρ=0.51323, df = 92, P<0.0001). Weight of the wax within the colony was taken out of the colony unit and weighed with each post mortem analysis. Weight of wax did not differ by treatment (2012: $F_{2, 88}$=0.97, P=0.3848). Data was also not significantly different when compared between sites

site ($F_{2, 56.33}$=0.27, P=0.7658). In both years, the number of bees that remained in the colony were counted with each post mortem analysis and compared to the colony's site and treatment. In 2011, although the P value was less than 0.05, further analysis by a Tukey Kramer post hoc determined no significant differences are warranted. In 2012 more dead, adult bees were left in the colonies with sun treatments than the colonies with two shade treatments (Figure 1.34), (2011: $F_{4, 31}$=5.85, P=0.0013, 2012: $F_{2, 97}$=5.95, P=0.0037).

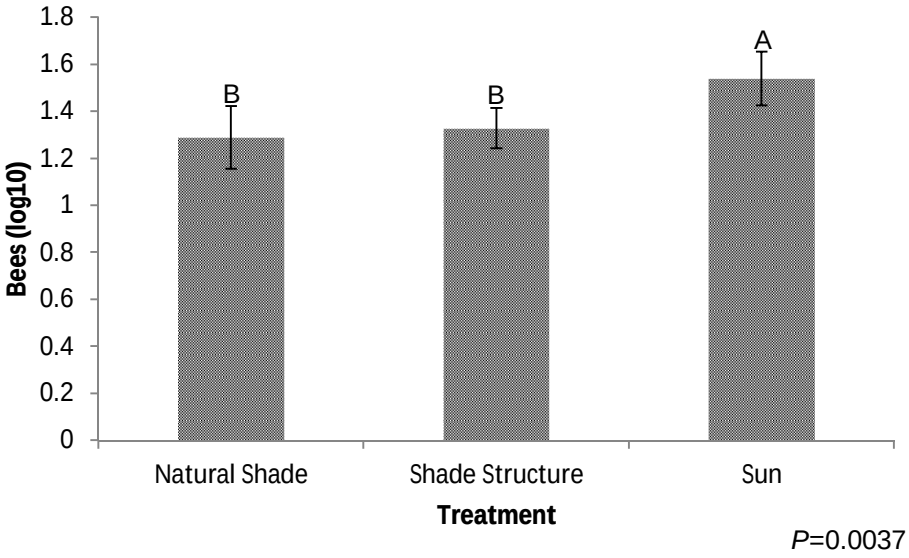

Figure 1.34. 2012 Watermelon, dead adult bees by colony treatment (log10 transformed). Error bars designate 95% confidence intervals and letters designate significant differences as determined by a Tukey Kramer post hoc test.

No differences were detected by field in 2011 (Figure 1.35), but differences were seen in 2012 (Figure 1.36), with field 3 having more bees than the other two, (2011: $F_{2, 33}$=1.0, P=0.3795, 2012: $F_{2, 97}$=6.571, P=0.0021).

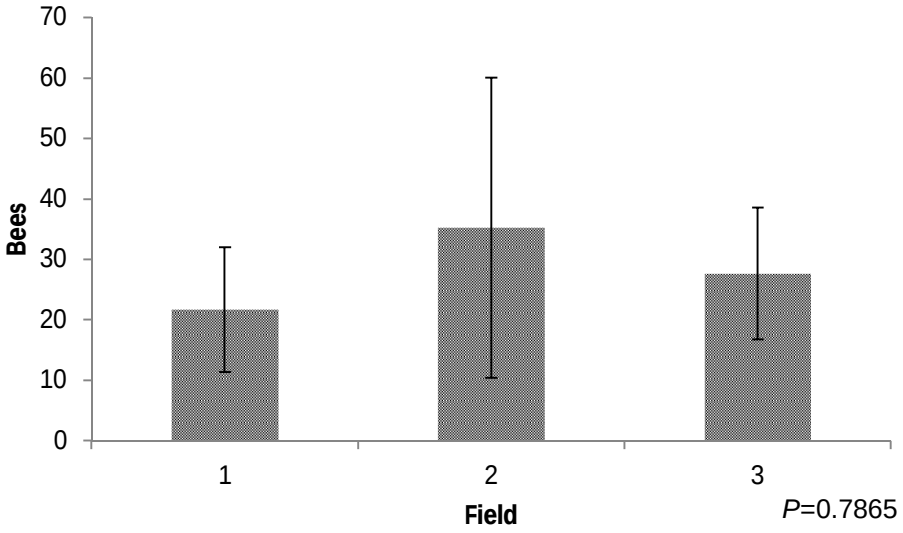

Figure 1.35. 2011 Watermelon, dead adult bees by field. Error bars show 95% confidence intervals

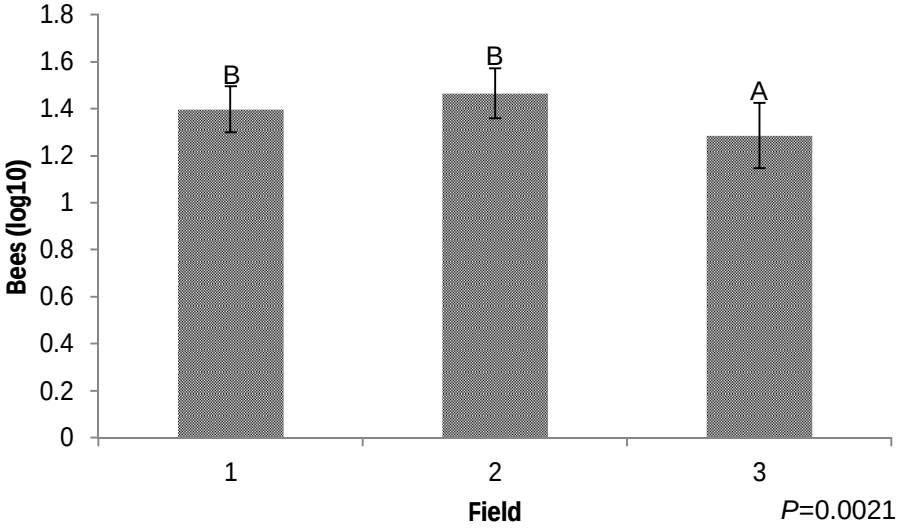

Figure 1.36. 2012 Watermelon, dead adult bees by field (log10 transformed), +/- 95% confidence intervals. Letters designate significant differences as determined by a Tukey Kramer post hoc test.

1.3.2.4 Colony sacrifice

The highest and lowest weighing colonies were sacrificed in the middle of the 2012 field season. These colonies were subsequently categorized as 'high' or 'low' based on their weight. These categories were then tested to determine if weight is an indicator of productivity. The high and low category were compared to mean total foraging, ($F_{1, 4}$=0.49, P=0.5231) and to mean number of cells found during the colony post mortem analyses. The higher weight colonies had more cells than the lower weight colonies ($F_{1, 4}$=37.02, P=0.0037).

1.3.2.5 Colony transfer

As explained above, each watermelon field had three quads of two different treatments and a third treatment with four quads. The four-quad treatment was different for each field. The extra fourth treatment quad from each field was transferred to the first pickling cucumber field a month into the study. Transferred quads were compared to non-transferred quad to test for differences in productivity. Colonies within the transferred quads had higher average weights throughout the season than non-transferred colonies ($F_{1, 112}$=5.39, P=0.0221). No difference was detected with wax weight in the post mortem analysis, ($F_{1, 110}$=1.96, P=0.1641). Non transferred colonies had more cells than transferred colonies, ($F_{1, 112}$=473.44, P<0.001).

1.3.2.6 In field forage

In 2012, honey bees and bumble bees were followed for 60 seconds, and the number of flowers they visited within that time was recorded and compared. Bumble bees visited more flowers in a 60 second period than honey bees ($F_{1, 66}$=17.22, P<0.0001).

1.3.2.7 Pollen

In 2012, 18 out of 73 (25%) pollen samples taken from pollen baskets of bees placed within the watermelon fields contained watermelon pollen. The most common pollen grains and the percentage of which they were detected within the watermelon fields were English plantain (57%), crown vetch (37%), and bitter sweet (36%). Separate statistical analyses were conducted on the detection of watermelon pollen in a sample and the number of watermelon grains found in a sample. No differences were found between the detection of watermelon pollen in samples of foragers along the field edge (natural shade treatments) and colonies within the field (shade structure and sun treatments) (P=1) or when testing the number of watermelon pollen grains by colony treatment ($F_{2, 70}$=1.07, P=0.35).

1.3.2.8 2011 Survey

The number of fruit set detected throughout the season was compared between fields with commercial honey bees and fields with commercial bumble bees. No statistical differences were reported ($F_{1, 665}$=0.17, P=0.6838). The number of aborted fruit were compared between fields with different bees with a one-way anova ($F_{1, 665}$=2.52, P=0.1129). The week that the first fruit set was detected was compared

between the fields with different pollinators ($F_{1, 16}$=0.19, P=0.6676). Finally, each survey's fruit set was compared to the number of weeks from planting in which it was counted. Differences were seen only between weeks from planting. The bee type did not yield significant differences nor did the interaction term (bee type: $F_{11, 660}$=0.03, P=0.8535, weeks: $F_{11, 660}$=30.01, P<0.0001, bee x weeks: $F_{11, 660}$=1.87, P=0.1138).

1.3.2.9 2012 Survey

Watermelon surveys were conducted twice in each field in 2012. One plant was chosen at 5 different distances per quad. The total number of fruit counted during both surveys was compared by distance the chosen plant was from quad (number of fruit on a plant 10 meters from quad, 20 meters, 30, 40, 50) and by the number of fruit counted on plants in each field. Difference was detected by site only, where more watermelon in survey 1 were counted at field 2 than the other fields (distance from quad: $F_{16, 268}$=0.33, P=0.8575, site: $F_{16, 268}$=1.43, P=0.0015, distance x site: $F_{16, 268}$=4.48, P=0.0185). Analyzing the total number of fruit counted by distance from quad in the first survey only, the different watermelon fields where the surveys were conducted were statistically different. Again, field 2 had more watermelon counts than the other fields, but the interaction term and distance from quad were not statistically significant (distance from quad: $F_{14, 135}$=0.32, P=0.8634, site: $F_{14, 135}$=11.19, P<0.0001, distance x field: $F_{14, 135}$=1.46, P=0.1790). No significant results were reported during the second survey that was conducted just before the first harvest (distance from quad: $F_{14, 120}$=0.17, P=0.09521, site: $F_{14, 120}$=0.77, P=0.4674, distance x field: $F_{14, 120}$=0.71, P =0.686). The same test was run and broken down by treatment

and no significant interactions were detected (survey 1: distance from quad: $F_{14, 135}$=0.28, P=0.8901, treatment: $F_{14, 135}$=2.99, P=0.0538, distance x treatment: $F_{14, 135}$=0.84, P=0.567; survey 2: distance from quad: $F_{14, 120}$=0.17, P=0.9516, treatment: $F_{14, 120}$=1.27, P=0.2843, distance x treatment: $F_{14, 120}$=0.69, P=0.7003).

1.3.2.10 2012 Harvest

In one of the three fields a watermelon harvest experiment was run. There was no difference detected in weight of the total harvest within the delineated transect by treatment ($F_{2, 92}$=2.38, P=0.0979). Analyzing each harvest separately also did not yield any significant results (harvest 1: $F_{2, 35}$=1.36, P=0.2791, harvest 2: $F_{2, 29}$=0.91, P=0.4136, harvest 3: $F_{2, 22}$=0.80, P=0.4629).

1.3.2.11 Temperature and relative humidity

In 2012, average weekly temperature (Figure 1.37) and relative humidity (Figure 1.38) of the colonies did not differ in colonies with different treatments, (temperature: $F_{2, 61}$=0.34, P=0.715, relative humidity: $F_{2, 20}$=3.30, P=0.0579).

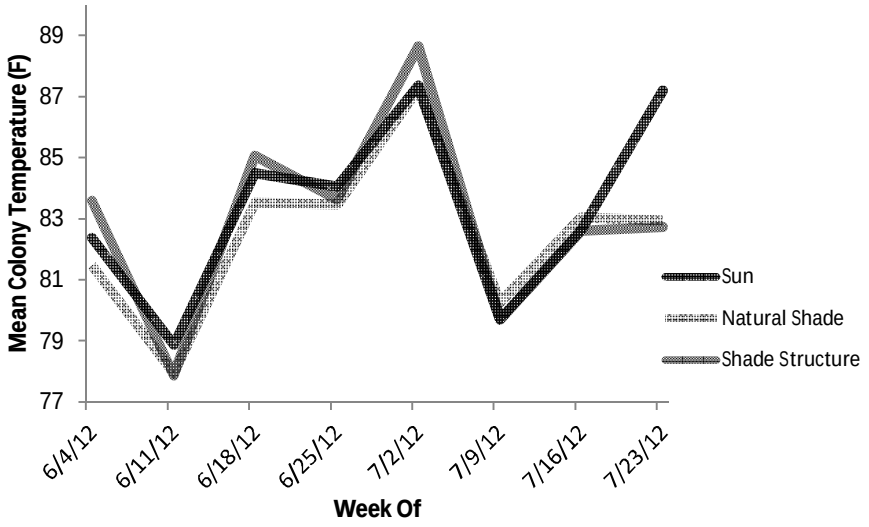

Figure 1.37. 2012 Watermelon, weekly average temperature by colony treatment.

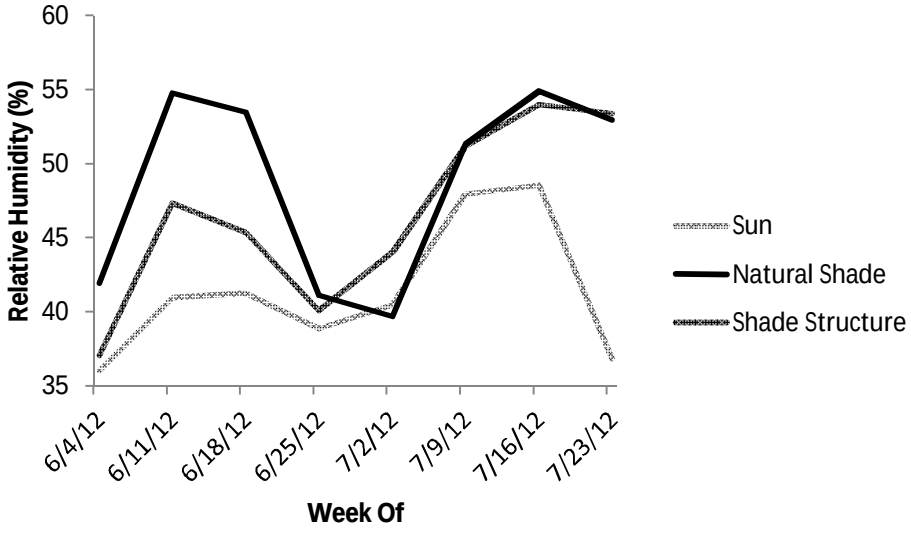

Figure 1.38. 2012 Watermelon, weekly average relative humidity by colony treatment.

1.3.3 Pickling cucumber

1.3.3.1 Five minute foraging counts

In 2012, bumble bee quads placed in pickling cucumber fields were not consistently observed foraging and due to the lack of recorded data statistical analyses were not possible. The following results in this section exclusively reflect data gathered in 2011 on pickling cucumber fields.

Peak foraging of an individual colony was determined and compared against treatment and site and found to be significant in both analyses. The peak recorded foraging trips were higher in colonies under a reflective A-frame shade structure than the reflective pentagon shade structure and the sun (Figure 1.39). Whereas field number two had a higher number of peak forage trips than the first field (Figure 1.40), (treatment: $F_{4, 29.5}$=7.81, P=0.0002, field: $F_{1, 78}$=12.38, P=0.0007).

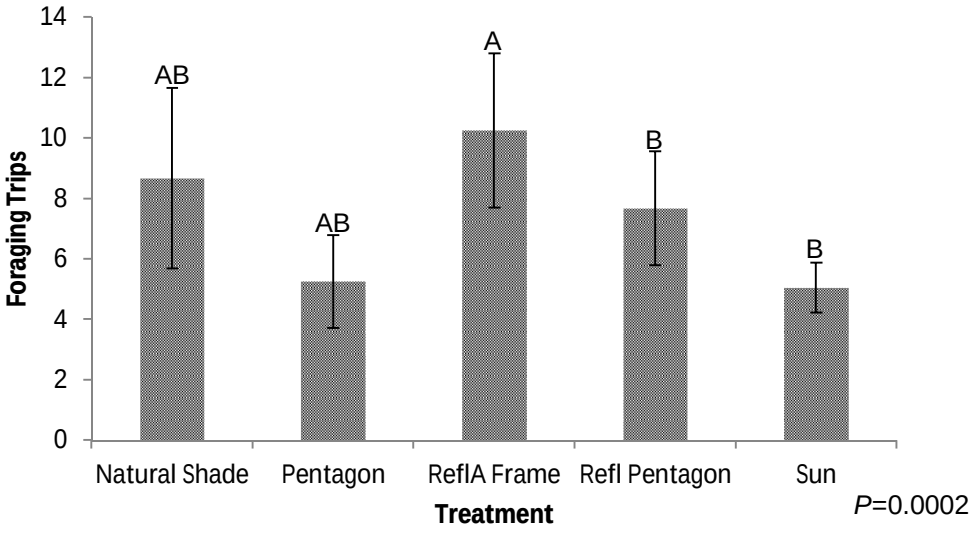

Figure 1.39. 2011 Pickling cucumber, peak forage by colony treatment +/- 95% confidence intervals. Letters denote statistical difference as determined by a Dunnett's T3 post hoc test.

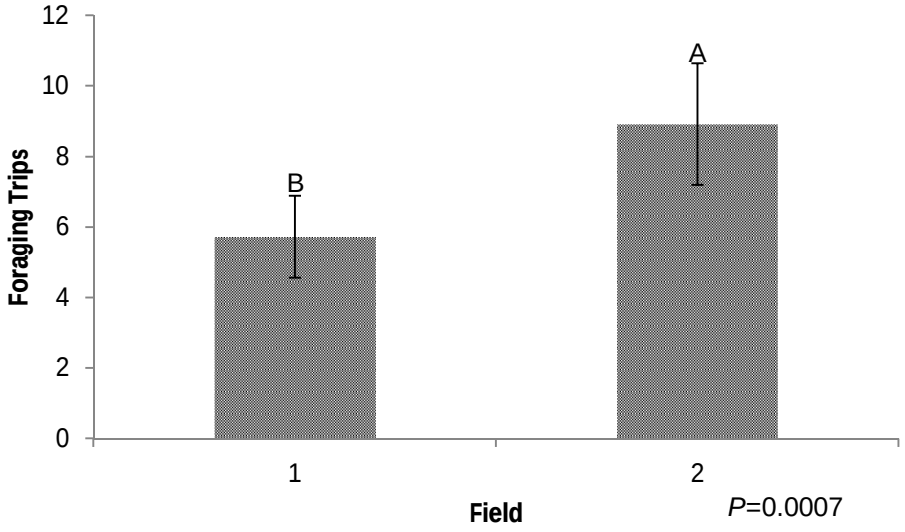

Figure 1.40. 2011Pickling cucumber, peak forage by field. Error bars denote 95% confidence intervals and letters designate significant differences as determined by Tukey Kramer post hoc test.

The date in which each colony reached its peak forage was not analyzed because the data did not meet assumptions of both parametric and non-parametric tests. Both reflective shade structures had higher average colony foraging trips than the colonies placed in the sun. Field two also had a higher number of average foraging trips than field one (treatment: $F_{4, 30.14}$= 7.01, P=0.0004, field: $F_{1, 78}$=13.26, P=0.0005). The reflective A-frame shade treatment was the only treatment that was significantly different from the sun treatment in total season forage. The same test was performed by site but no differences were detected (treatment: $F_{4, 75}$=3.37, P=0.0137, field: $F_{1, 78}$=3.79, P=0.0552).

1.3.3.2 Weight

Tests describing weight and weight changes in colonies in the cucumber fields compared weight by treatment and site in 2011. In 2012, all cucumber colonies were placed at the same field and moved to a second field after the harvest of the first. Therefore, in 2012, statistical tests trying to delineate differences in site are not possible. In 2011, all shaded structures had higher average weight of colony through season than sun. The natural shade had the highest average weight, of all treatments (2011: $F_{4, 31.09}$=43.49, P<0.0001, 2012: $F_{1, 38}$=1.23, P=0.2748). The same test was run when looking at differences between sites in 2011, with field 2 having a higher average, average weight than field 1. ($_{F1, 78}$=4.51, P=0.0368). Change in weight from the beginning of the season to the peak was measured. When compared to treatment, the test could not be statistically tested for either year. The 2011 data did not match any of the assumptions of the appropriate parametric or non-parametric tests. Additionally, in only a few instances in 2012 did an increase in weight occur from the first day. Most colonies were in weight decline the day they were placed in the field, making most of the values in this collection zero. Change in weight by site was able to be analyzed in 2011 only and yielded statistically significant differences of weight gain by field (Figure 1.41), ($F_{1, 78}$=6.73, P=0.0113), where the means of field 2 were higher than the means of field 1.

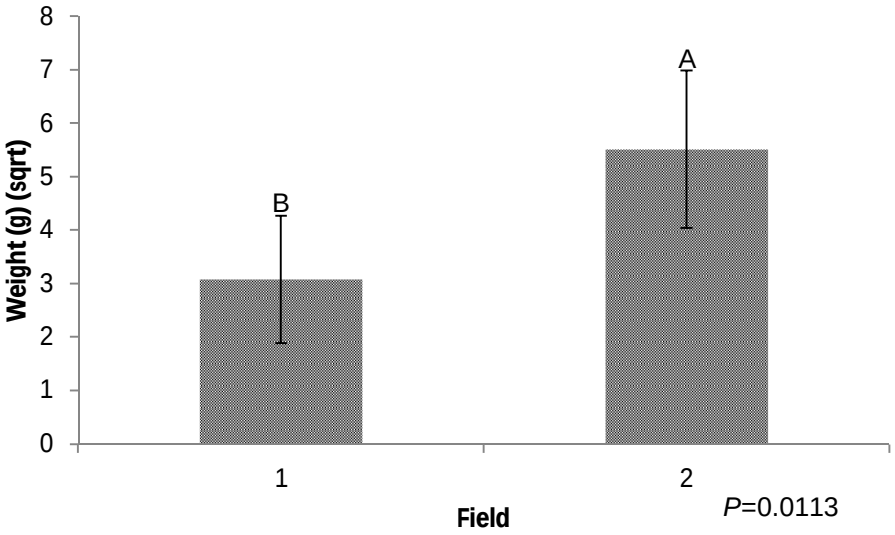

Figure 1.41. 2011 Pickling cucumber, weight change from start to peak weight by field +/- 95% confidence intervals. Letters denote statistical significance as determined by a Tukey Kramer post hoc test.

All shade treatments with the exception of natural shade had higher peak weights than the sun treatment in 2011 (Figure 1.42), ($F_{4, 28.68}$=23.39, P<0.0001) and no differences were detected in 2012 (Figure 1.43), ($F_{1, 38}$=1.12, P=0.2972).

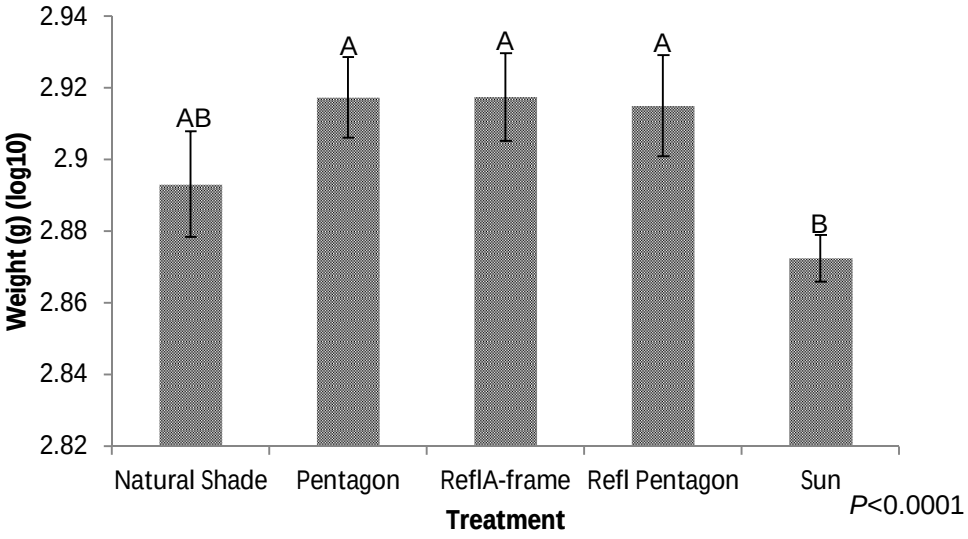

Figure1.42. 2011 Pickling cucumber, peak weight by colony treatment +/- 95% confidence intervals. Letters designate significant differences as determined by Dunnett's T3 post hoc test.

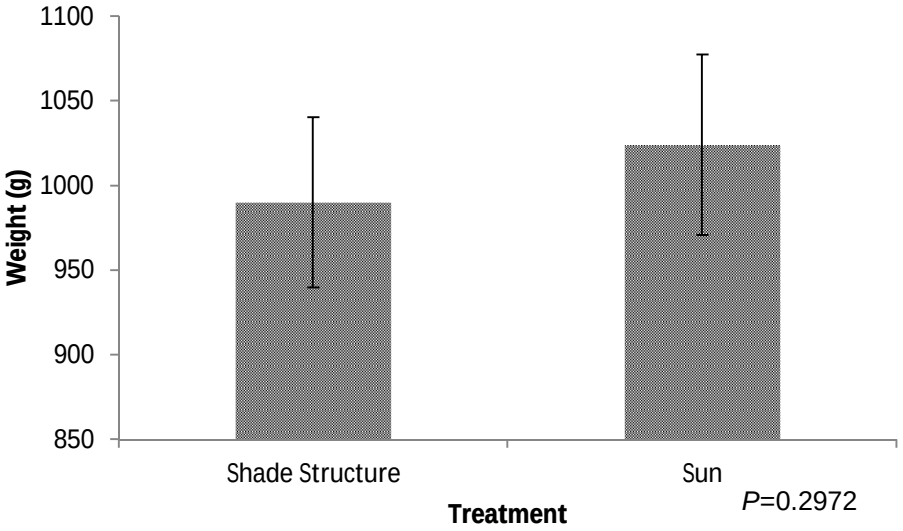

Figure 1.43. 2012 Pickling cucumber, peak weight by colony treatment +/- 95% confidence intervals.

Peak weight by field was significant in 2011, the only year where the test is applicable (Figure 1.44), ($F_{1, 78}$=8.09, P=0.0057).

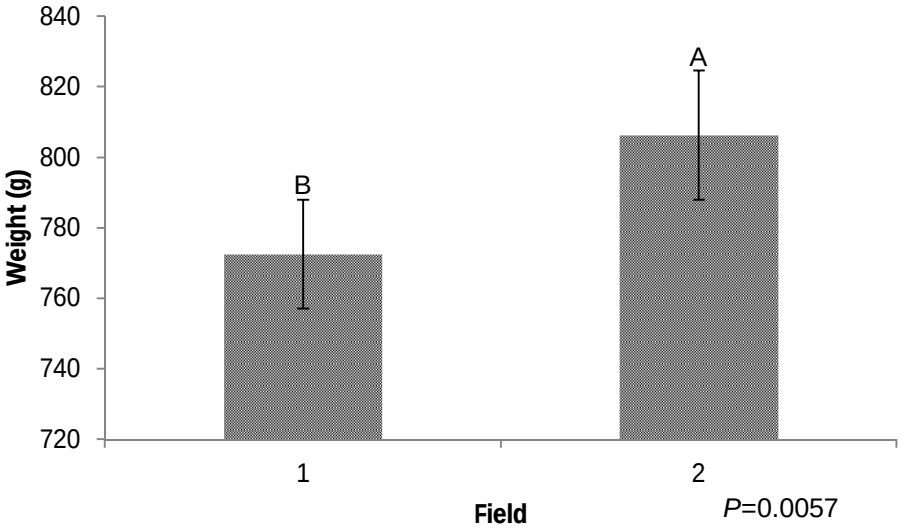

Figure 1.44. 2011 Pickling cucumber, peak weight by field +/- 95% confidence intervals. Letters designate significant difference as determined by a Tukey Kramer post hoc test.

The date in which the 2011 cucumber colonies reached their peak was not able to be analyzed with treatment as the data did not meet assumptions of both the parametric and non-parametric tests. When analyzed by field, the mean number of days it took to reach peak weight in field 2 was higher than field 1 (Figure 1.45), ($F_{1, 78}$=6.36, P=0.0137). Again this was unable to be tested in 2012.

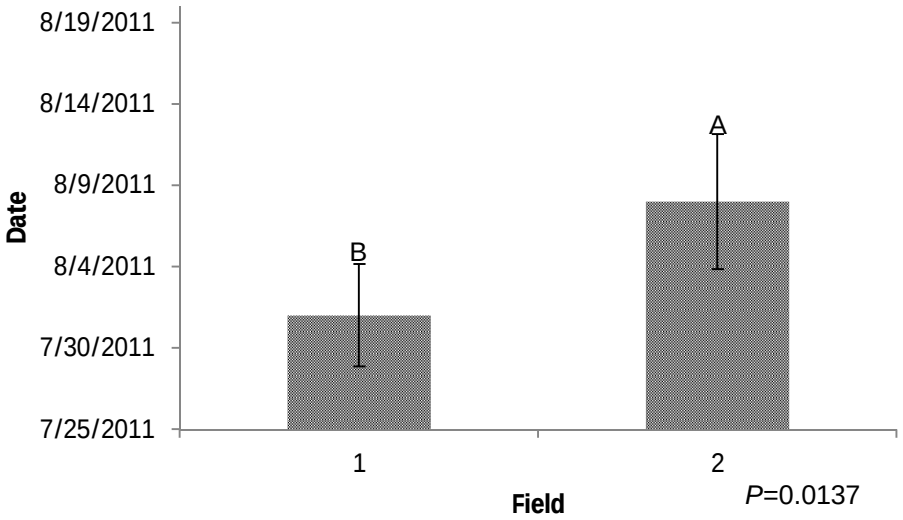

Figure 1.45. 2011 Pickling cucumber, peak weight date by field +/- 95% confidence intervals. Letters designate significant differences as determined by Tukey Kramer post hoc test.

1.3.3.3 Post mortem analyses

The total number of cells found in each colony was compared to the treatment that the colony was given. In 2011, the pentagon shade structure had more cells than the sun treatment (Figure 1.46). No difference was detected in 2012 (Figure 1.47), (2011: $F_{4, 22}$=5.52, P=0.0031, 2012: $F_{1, 38}$=1.79, P=0.1891).

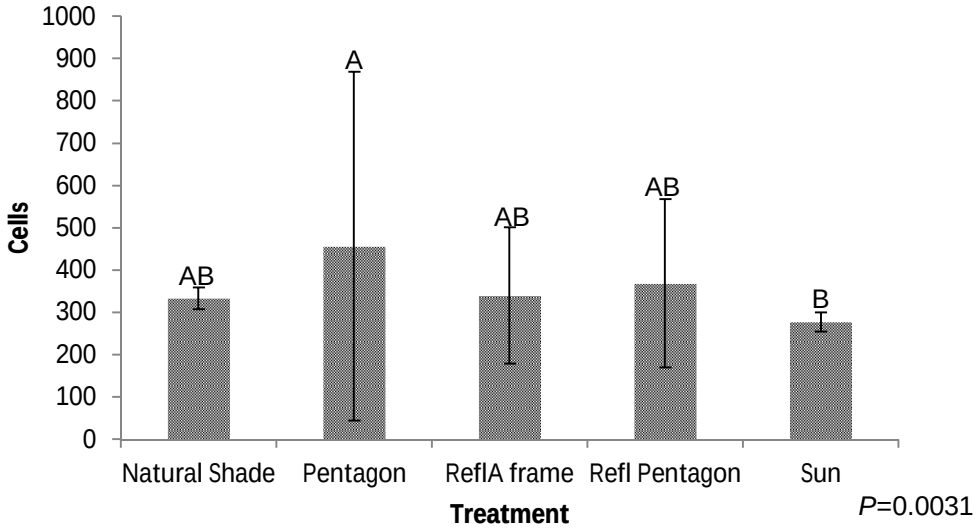

Figure 1.46. 2011 Pickling cucumber, total cells by colony treatment +/- 95% confidence intervals. Letters denote significant differences as designated by a Tukey Kramer post hoc test.

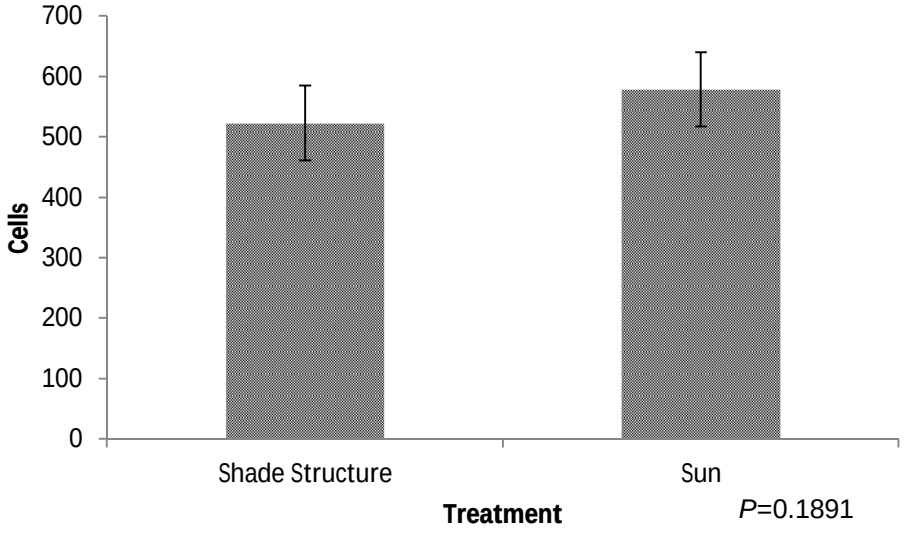

Figure 1.47. 2011 Pickling cucumber, total cells by colony treatment +/- 95% confidence intervals.

The same test was analyzed by field for the year 2011 (Figure 1.48), ($F_{1, 25}$=0.04, P=0.8496).

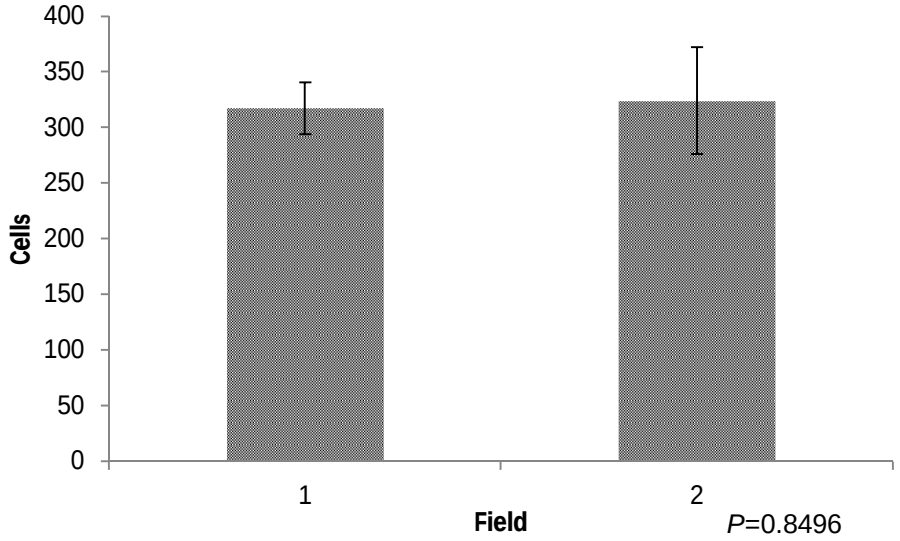

Figure 1.48. 2011 Pickling cucumber, total cells by field, +/- 95% confidence intervals.

Cells were further classified into queen cells and non-queen cells. When testing non-queen cells by treatment, the pentagon shade structure had significantly higher amounts of non-queen cells of all treatments but the reflective pentagon shade structure. No difference was seen in 2012 (2011: $F_{4, 34}$=6.50, P=0.0005, 2012: $F_{1, 38}$=3.21, P=0.0814). In 2011, non-queen cells were compared to the site in which the bees were placed ($F_{1, 37}$=0, P=0.9472). The number of queen cells by treatment was tested and no statistical significance was found either years (2011: $F_{4, 35}$=1.89, P=0.134, 2012: $F_{1, 38}$=3.72, P=0.0784). The number of queen cells by site were analyzed with the 2011 data and no differences were detected between the two fields, ($F_{1, 38}$=0.03, P=0.8739). Peak colony weight during the season was compared to the total number of cells (2011: ρ=0.28994, df=37, P=0.0734, 2012: ρ=0.19205, df=18

P=0.2351). Average colony weight throughout the season versus total number of cells yielded significant results only in 2011 (Figures 1.49, 1.50), (2011: ρ=0.42482, df=37 *P*=0.007, 2012: df=92, r^2=0.0827, *P*=0.0719).

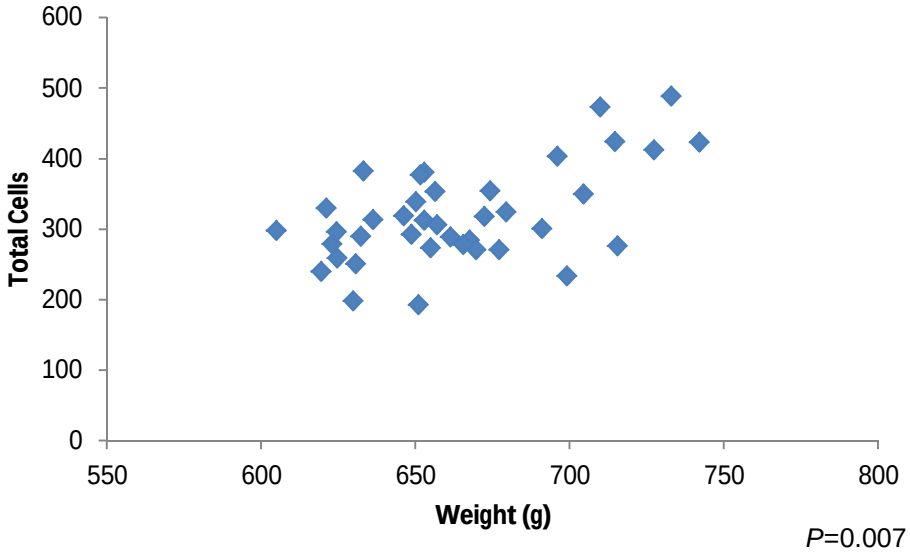

Figure 1.49. 2011 Pickling cucumber, total cells vs mean weight, analyzed with a Spearman's Rank Correlation due to heteroscedasticity.

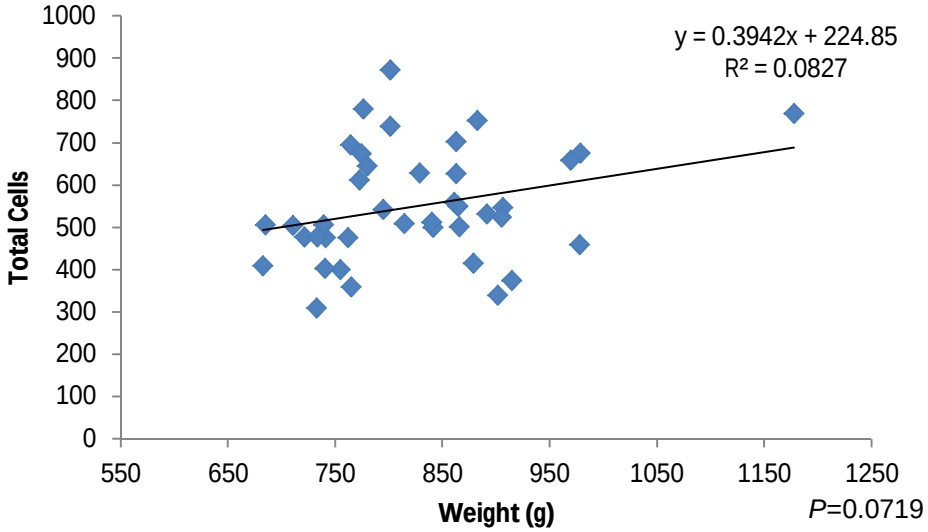

Figure 1.50. 2012 Pickling cucumber, total cells vs mean weight.

The last recorded weight of the colony at the end of the season was compared to the total number of cells counted within the colony during post mortem analysis (2011: ρ=0.32165, df=37, *P*=0.0458, 2012: ρ =0.26583 df=37, *P*=0.1019). The measured weight of the wax within the cell during post mortem analysis was measured. In 2011 all shade structure treatments had significantly higher weighing wax than the sun treatment (Figure 1.51), ($F_{4, 44}$=7.10, *P*=0.0002). In 2012, the sun treatment colonies had higher weighing wax than the shade structure colonies (Figure 1.52), ($F_{1, 29.71}$=4.26, *P*=0.0458).

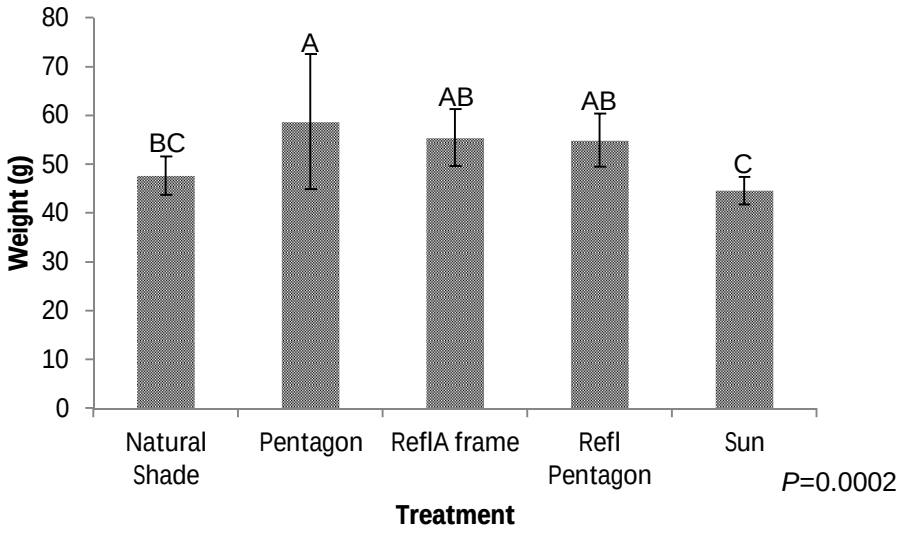

Figure 1.51. 2011 Pickling cucumber, weight of wax by colony treatment +/0 95% confidence intervals. Letters designate significant difference as determined by a Tukey Kramer post hoc test.

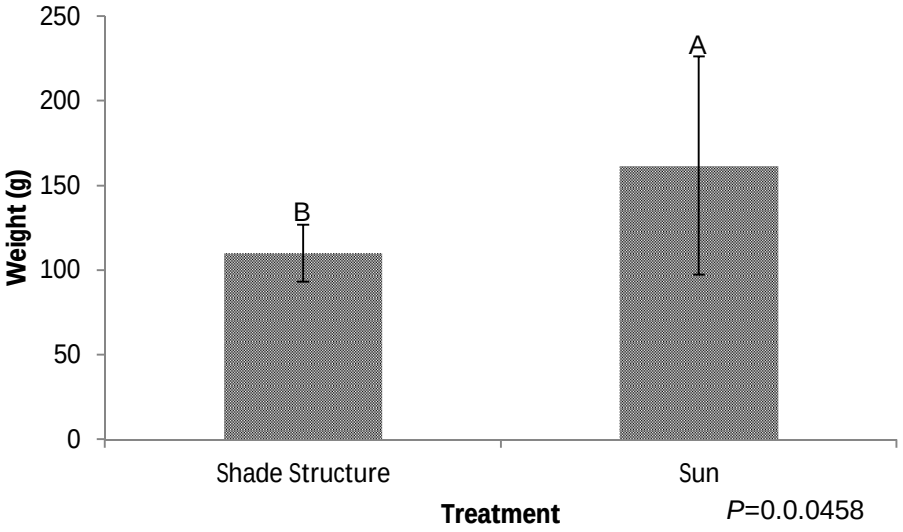

Figure 1.52. 2012 Pickling cucumber, weight of wax by colony treatment +/- 95% confidence intervals. Letters designate significant differences as determined by Dunnett's T3 post hoc test.

Weight of wax per site was calculated in 2011 with no found differences (Figure 1.53), ($F_{1, 47}$=0.12, P=0.7348).

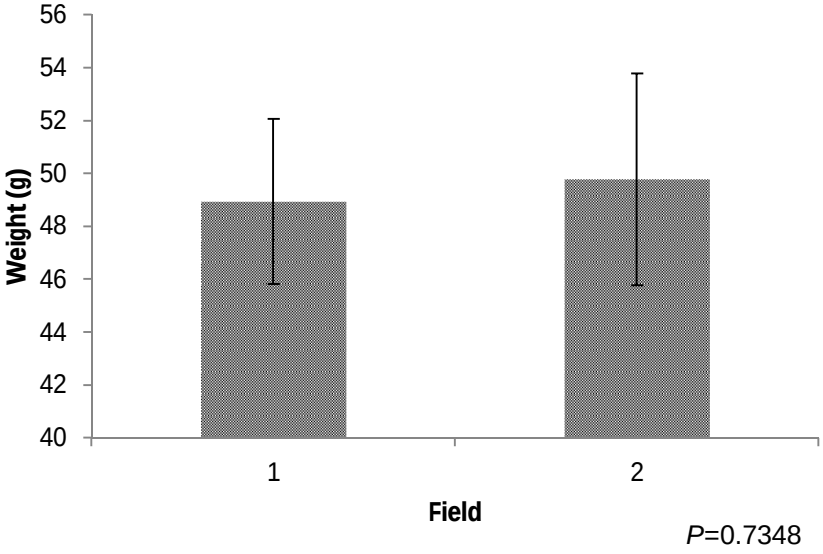

Figure 1.53. 2011 Pickling cucumber, weight of wax by field +/- 95% confidence intervals.

Finally, the number of dead bees left in the colony were counted and compared against treatment and site. The number of bees compared to the treatment was not found to be significant in either year (2011: $F_{4, 25.88}$=1.26, P=0.2954, 2012: $F_{1, 38}$=2.9, P=0.097). In regards to site, field 2 had a higher number of dead adult bees left in the colony than field 1 ($F_{1, 74}$=4.01, P=0.0489).

1.3.3.4 Insects within the field

In 2011, 168 pollinators were caught during the sweep net study, 132 of them were Apidae-*Apis.* Apidae-*Bombus* was the next most abundantly caught taxa with 17, followed by Syrphidae with 8. In 2012, out of 75 pollinators caught by sweep net, 65 were Apidae-*Apis.* The next largest amount of a pollinator taxa caught were Syrphidae. No Apidae-*Bombus* were caught. In the bowl traps, 84 pollinators were caught. Apidae-*Apis,* Halictidae, and Syrphidae were the most abundant taxa with 27, 20, and 20 individuals caught, respectively. Out of the 84 pollinators, 6 Apidae-*Bombus* were recorded, the other Hymenopterans were, Apidae-*Melissodes,* and Vespidae.

1.3.3.5 Harvest

In 2011 pickling cucumbers were harvested and weighed based on USDA pickling cucumber grades. They were harvested at four different location categories (middle of the field close to bees, middle of the field away from bees, edge of the field close to bees, edge of the field away from bees). The weight of all oversize, 4a, 2a,

1b, and 1a grades by location were not analyzed due to insufficient occurrences of these grades during those harvests. The total weight of pickling cucumber grades 3b, 3a, 2b, and culls and nubs that were harvested were compared against the transect in which they were harvested (e.g. all of the 3b pickling cucumbers that were harvested were analyzed against the transect in which they were picked – middle of the field close to bees, middle of the field away from bees, edge of the field close to bees, edge of the field away from bees). The 3b grade by transect type was the only pickling cucumber grade to have statistically different weights in different harvest locations of the field. The middle area with bees was significantly higher than the middle area without bees, but did not differ significantly from either of the edge treatments (3b: $F_{3, 13}$=5.31, P=0.0131).

The remaining treatments did not have any statistically significant results (3A: $F_{3, 14}$= 0.57, P=0.6415, 2B: $F_{3, 14}$=1.53, P=0.2503, culls and nubs: $F_{3, 15}$=0.33, P=0.8036). When comparing the harvest weights between the two fields (field 1 had placed bumble bees only, and field 2 had both bumble bees and honey bees), only the culls and nubs and the 3a grades had significant differences between the fields. In both cases, field 1 had higher average weights than field 2 (culls and nubs: $F_{1, 17}$=13.06, P=0.0021, 4A: $F_{1, 6}$=3.62, P=0.1057, 3B: $F_{1, 15}$=0.86, P=0.3697, 3A: $F_{1, 16}$=20.19, P=0.0004, 2B: $F_{1, 16}$=2.85, P=0.1107, 2A: $F_{1, 13}$=1.74, P=0.2102, 1B: $F_{1, 15}$=0.25, P=0.6215, 1A: $F_{1, 11}$=0.50, P=0.4924).

In 2012, pickling cucumbers were categorized by if they were normal shaped (indicating adequate pollination) or culls and nubs (fruit that did not develop into a

normal shape because of inadequate pollination). The number and weight of all pickling cucumbers in each category were recorded. No difference was detected when comparing the weight and number of culls by treatment (weight: $F_{1, 6}$=0.93, P=0.3713, number: $F_{1, 6}$=0.32, P=0.5902). Further, no differences were seen with the weight and number of non culls by treatment, (weight: $F_{1, 6}$=1.62, P=0.2498, number: $F_{1, 6}$=0.60, P=0.4675).

1.3.3.6 Temperature and relative humidity

No data is available for temperature and relative humidity in pickling cucumbers due to temperature recorders that failed to record and/or upload successfully.

1.3.4 Pesticides

Table 1.1. List of pesticides detected in bees and wax samples in ppb concentrations in 2011. All values in parentheses indicate honey bee LD50 from PPDB unless otherwise indicated. - = no detection, 2 = Thompson 2001, 3=Mullin et al. 2010, / = bumble bee LD50.

Crop	Watermelon		Pickling Cucumber		Strawberry	
Field	**3**		**2**		**1**	
Substrate	**Bees**	**Wax**	**Bees**	**Wax**	**Bees**	**Wax**
Chemical (concs in ppb) (LD50)						
Acetamiprid *(8,090) 48 hour contact*	-	-	-	-	-	12.5
Azoxystrobin *(25,000) 48 hour oral*	22.7	168	56.6	313	-	-
Carbendazim *(50,000) 48 hour contact*	-	-	13.1	40.6	10.9	72.4
Chlorothalonil *(40,000) 48 hour oral*	312	4860	-	1240	-	-
Chlorpyrifos *(59) 48 hour contact/ (11,400)*[2]	-	-	-	3.7	-	-
Coumaphos *(46,300)*[3]	-	29.7	-	48.4	-	24.9
Cyfluthrin *(1) 48 hour contact*	-	-	4.9	-	-	-

Cyprodinil *(113,000) 48 hour oral*	45.8	39.8	-	-	57.1	39.4
Difenoconazole *(>100,000) 48 hour contact*	266	-	-	-	138	-
Fludioxonil *(>100,000) 48 hour contact*	-	-	-	-	47.2	75.2
Fluvalinate *(not available)*	-		-	8	-	-
Hydroxychlorothalonil *(not available)*	-	226	-	-	-	-
Methomyl *(800)[2]/(3,200)[2] 24 hour oral*	-	-	13.5	12	-	-
Phosmet *(220) 48 hour contact*	-	-	-	-	-	38.1
Pyraclostrobin *(>73,100) 48 hour oral*	-	-	-	-	46.1	120
Tebuconazole *(>83,050) 48 hour contact*	40.3	49.2	-	-	-	-
Tebufenozide *(> 234,000) 48 hour contact*	-	-	-	-	73	153
Thymol *(>200,000) 48 hour contact*	62.8	71.8	-	-	-	-

Table 1.2. List of pesticides detected in bees and wax samples in ppb concentrations in 2012. All values in parentheses indicate honey bee LD50 from PPDB unless otherwise indicated. - = no detection, 2 = Thompson 2001, 3=Mullin et al. 2010, / = bumble bee LD50.

Crop	Watermelon		Pickling Cucumber		Strawberry	
Field	**3**	**3**	**2**	**2**	**1**	**1**
Substrate	**Bees**	**Wax**	**Bees**	**Wax**	**Bees**	**Wax**
Chemical (concs in ppb) (LD50)						
Azoxystrobin *(25,000) 48 hour oral*	-	-	73.4	-	103	2970
Bifenthrin *(150)[3]*	7.8	-	-	-	-	31.2
Boscalid *(1,550,000)[3]*	-	-	-	-	70	79.5
Captan *(100000) 48 hour oral*	-	-	-	-	-	2440
Carbendazim *(>500,000) 48 hour contact*	-	-	-	-	-	20.1
Chlorfenapyr *(120) 48 hour exposure type not reported*	2.8	-	-	-	-	-
Chlorothalonil *(40,000) 48 hour oral*	5710	48700	-	34400	-	178
Chlorpyrifos *(59) 48 hour contact/(11,400)[2]*	-	6.2	-	-	5.1	25.5
Coumaphos *(46,300)[3]*	-	-	-	-	-	13.1
Cyprodinil *(113,000) 48 hour oral*	72.4	11.3	-	-	32.5	43.7
Fenbuconazole *(1,490,000)[3]*	-	-	54.8	-	-	16.9
Fenpyroximate *(15,800) 48 hour contact*	6.3	-	-	-	-	-
Fluvalinate *(15,860)[3]*	-	2.5	-	-	-	-
Hydroxychlorothalonil *(not available)*	-	196	-	-	-	-
Methoxyfenozide*(>100,000) 48 hour oral*	-	-	60	-	-	-
Pendimethalin *(100,000) 48 hour contact*	16.6	40.4	-	-	45.9	43.3

Phosmet *(220) 48 hour contact*	-	-	-	-	17.1	129
Pyraclostrobin *(>73,100) 48 hour oral*	-	-	-	-	11.2	19.9
Tebuconazole *(>73,100) 48 hour oral*	21.1	12.7	47	81.1	-	-
Tebufenozide *(>234,000) 48 hour contact*	-	-	-	-	-	103
Trifloxystrobin *(>200) 48 hour oral*	-	-	-	-	-	49.3

1.4 Discussion

1.4.1 Bumble bee biology

The variety of placement treatments including the various designs of the shade structures provided by Koppert Biological Systems caused differences in the performance of various metrics assessed during the field season. These metrics tested colony productivity by determining forage rates, colony weight and cell production within the colony. All treatment placements generally provided a condition for the colony that was superior to a sun placement in relation to productivity tests results. These results align with the need for bumble bees to maintain their nest temperature at 30°C. This temperature is maintained by bumble bees producing heat at low ambient temperatures or fanning the nest when the weather gets too warm (Goulson 2010, Heinrich 2004, Vogt 1986). These temperature-regulating actions require energy, thus, energy spent regulating temperature is not available to optimize colony productivity (Heinrich 2004). The less energy available decreases the allotment of energy for nectar and pollen foraging and overall reduces intrinsic worth to growers. Indeed, in most five minute foraging counts, weight analyses, and nest post mortem analyses, the shaded colonies foraged more, weighed more, or performed in a way that indicated higher productivity, than colonies placed in the sun. Further, the more worker bees

needed to thermoregulate a colony, equates to less bees available for brood maintenance or other duties needed for the colony (Vogt 1986).

Mid-season colony sacrifice determined that overall weight was an appropriate measure of productivity. The highest weighing colonies in each watermelon field had significantly more cells in a post mortem analysis than the lowest weighing colonies. Further, peak weight was positively correlated to the total number of cells in the colony and end weight and average weight were significantly correlated to total cells. Cells are produced in a colony to house brood, pollen and nectar. Having more brood to nourish will force a colony to not only build more cells but to visit more flowers in order to collect more pollen and nectar. This pollen and nectar collection will intuitively increase weight of the colony and more importantly, will hopefully help to pollenate more flowers, increasing productivity for growers. This supported our hypothesis that weight can be an indicator of colony productivity.

Interestingly, we found that when counting dead, adult bees in the post mortem analyses, sun treated colonies left more bees than their shaded counterparts. Perhaps this is evidence that energy expended for colony hygienic behavior decreases when more energy is needed to maintain colony temperatures. In a healthy colony, dead, adult bees are most likely removed to reduce inevitable occurrences of disease when chronically exposed to the dead (Cremer et al. 2007). Evidence of dead immature bee removal was witnessed throughout the experiments, especially after delivery of the commercial quads to the field. Larvae that did not survive the trip were removed by

worker bees and dropped outside of the colony. The removal of deceased adult bees is speculated but was not specifically witnessed during this project.

Colonies placed in the shade had significantly more non-queen cells than their counterparts in the sun in 2012 watermelon colonies. A similar trend was seen in the 2011 pickling cucumber colonies were pentagon shade structure colonies had significantly more non-queen cells than all other treatments. These colonies were devoting more energy and resources to the production of worker bees. An increase in number of workers should equate to more foraging bees.

Through these experiments we can conclude that commercial bumble bee units must be placed under some shade to increase longevity and overall productivity throughout the growing season. This statement is displayed in the watermelon temperature figure, Figure 1.37. This figure demonstrates the ability for bumble bees to thermoregulate their nests, despite the differences in sun exposure. However, as the season progresses the bumble bees in the sun stop regulating the temperature of their colony earlier in the season than the other two treatments. The temperature regulation stops presumably because the colony is no longer active and thus is no longer foraging. The temperature of the sun treated colonies increases while the other two treatments continue on, regulating the temperature of the colony.

Overall, the experiments testing colony productivity and colony placement presents much evidence that shaded colonies expend less energy and expend less general worker resources thermoregulating the colony, during the summer growing months. This relaxation from maintaining colony temperature allows for the energy to

be directed towards productivity as witnessed by the increase in foraging and weight in colonies placed in shade.

1.4.2 Bumble bee affinity to crops

The bumble bee's ability to transfer pollen has been studied many times and their efficiency at pollen transfer has been found to equal or exceed that of honey bees for specific crops. The bumble bee's ability to buzz pollinate, forage in harsh weather and earlier hours, and their longer tongues which allow them to reach deeper corollas makes them efficient and reliable pollinators (Goulson 2010, Heinrich 2004, Stanghellini et al. 1997, 1998, Velthuis and van Doorn 2006). Stanghellini and colleagues in 1997 1998, and 2002 showed that bumble bees transfer pollen in a way that is equivalent or surpasses the ability of the honey bees to do the same, in watermelon and cucumber crops. In our study, when compared to honey bees in the field, bumble bees foraged on statistically more flowers in a minute's time, therefore spreading pollen grains at a faster rate.

With this information, we decided to test what the bumble bees were actually foraging on in the field and dissected full pollen loads collected by foragers. Pickling cucumber was not tested in our experiment, as no pollen was collected by researchers in 2011, and in 2012, colonies were not viable because they were rarely observed foraging and were eventually overrun by honey bees. Strawberry pollen was found in nearly half of samples from bumble bees placed in a mixed orchard in a strawberry field, in 2011. The percentage decreased in 2012, where only 8% had strawberry pollen in the subsample of the dissected corbiculas. In 2011, some of the samples

were taken from colonies placed within high tunnels, and thus their foraging options were limited to only strawberry. We believe that this explains the differences in the preferences for strawberry between the two years. In the watermelon crop, 25% of the 73 samples tested contained watermelon pollen. Although strawberry and watermelon pollen were not the dominant pollen grains detected in these samples bumble bees were constantly and consistently detected in the fields throughout both seasons and found foraging on the watermelon and strawberry blooms. These foragers observed on the blooms very well could have been exclusively nectar foragers. However, if foragers are strictly on the crops for the intention of gathering nectar, transfer of pollen should still be occurring. Pollen samples tested from colonies with shade treatment were not statistically different from samples collected from sun treated colonies or were in field treatments (sun and shade structures) different from the natural shaded, edge treatments.

Further, there were statistically significant results during the watermelon survey analysis, where field number 2 consistently registered more fruit set counts than the other two fields. We believe that there are two reasons that this could have occurred. First, as field 2 had more quads with the shade structures, more productive colonies in the middle of the field could be allowing for more fruit set seen during the watermelon surveys. The second reason these statistical differences could have been detected, were the presence of honey bees in field number 2. There were at least two other watermelon fields that used commercial honey bees, less than a mile from field 2. Throughout the season, many of the bumble bee colonies in field 2 were invaded

by honey bees, and perhaps more honey bees made their way into the crop either adding more pollinators increasing pollination services to the crop, or perhaps it could be exemplifying honey bee superiority in watermelon. Throughout the season, we did notice honey bees in the other two fields, but we cannot be sure as to what concentration. During both years of our experiments honey bees were detected in our bumble bee only fields. The wide spread use of honey bees in all pollenated crops and their ability to forage in long ranges, makes it is nearly impossible to conduct a study that isolates honey bees from an open agricultural field. Therefore, we cannot accurately compare the ability of bumble bee and honey bees to pollenate the crops from our study.

In both years, bumble bees were not readily seen foraging within the pickling cucumber fields in which they were placed. Results from the sweep net and bowl trap studies in which small numbers of bumble bees were collected, and the failure of the pollen transfer study because of lack of bumble bee presence in the field, support the notion that commercial bumble bees do not readily forage on pickling cucumbers. In 2011, pickling cucumbers were placed in two fields, one with and one without commercial honey bee colonies. In both instances, honey bees were the dominant pollinator found in the field. Bumble bees were rarely collected and scarcely sited. In bumble bee colonies purchased for the 2012 pickling cucumber crop, bumble bees were scarcely detected foraging during the whole season. This led us to the conclusion that these particular units were not viable pollinators. Further, when the bumble bees were placed in the second pickling cucumber field after harvest of the

first, bumble bees were completely robbed out by honey bees also placed within the same field. After being invaded by honey bees, bumble bee colonies remained alive but were not observed foraging. Despite having little data from the 2012 pickling cucumber fields we do not believe that commercial bumble bees are a reasonable pollinator for the crop. However, based on the papers by Stanghellini and colleagues that give evidence of high seed set (1997, 1998), low fruit abortion (1997), and high pollen deposition (2002) in cucumbers pollenated by commercial bumble bees, we believe that further scrutiny in this system is warranted.

1.4.3 Pesticides

Pesticides were detected in the bees and the wax of each sample tested. LD_{50} were taken from Pesticide Properties Database (PPDB), a tool developed by the University of Hertfordshire in the UK, unless otherwise indicated. The LD_{50s} are for honey bee contact or oral exposure for 48 hours as bumble bee exposure has not been investigated to the same extent in which it has been explored in honey bees. We find these LD_{50s} inapplicable to our study for a few reasons. First, as bumble bees are larger than honey bees, they are known to consume more pollen and nectar on a daily basis than honey bees (EPA 2012). If pollen and nectar contain pesticide residue, bumble bees will likely come into contact with these chemicals at a faster rate, by the simple fact that adult bumble bees eat larger quantities per day than honey bees.

Table 1.3. Comparison of oral exposure to pollen and nectar for adult Apis and Bombus bees (EPA 2012).

Taxa	Nectar (mg/bee/day)	Pollen (mg/bee/day)	Total

Apis mellifera	292	0.04	292
Bombus spp.	183-372	27-30	210-402

Table 1.4. Comparison of oral exposure to pollen and nectar for larval Apis and Bombus bees (EPA 2012).

Taxa	Nectar (mg/bee/day)	Pollen (mg/bee/day)	Total
Apis mellifera	117	2.7	120
Bombus spp.	60	22-23	82-83

Second, in our study within the watermelon field, bumble bees foraged on more flowers per minute than honey bees. Therefore, bumble bees can be exposed to larger quantities of pesticides due to their increased visitation rates. Further, as stated above, the LD_{50s} are applicable for an exposure of just 48 hours (PPDB). In our experiment, the fields in which our bumble bees foraged were generally sprayed weekly; and the chemicals found in the substrate tested were from a season's worth of these weekly sprays. Thus, the commercial bumble bees are generally exposed to pesticides over a much longer time than is indicated in the given $LD_{50s.}$ Also there is growing evidence that exposure to multiple pesticides at a time creates synergisms leading to possible additive effects to honey bees. Much of the possibility for synergism has been shown between fungicides and neonicotinoids and pyrethroids (Johnson et al. 2010), all of which were found in the pesticide analysis. Bees are often exposed to more than one pesticide at a time within the field and are therefore subject to this multiple pesticide synergism. Much of these tests have been conducted in the

lab rather than in practical applications in the field and thus field studies testing synergism are warranted for the future (Iwasa et al. 2003, Johnson et al. 2009, 2010, Pilling and Jepson 1993). Currently, the US EPA is working on developing studies to test chronic honey bee exposure. At this time, there are no formal guidelines developed to conduct chronic toxicity tests on honey bees (EPA 2012), and to the best of our knowledge, there is no information available looking at bumble bee chronic exposure to these chemicals. Therefore, due to lack of scholarly literature and lack of funds that would allow for our own colony level pesticide analyses (rather than field) we have no real idea of the effects of the chemicals on the pollination ability of the commercial bumble bees within the field. Further, we call for such future analyses to be considered and conducted, as we believe not enough is known about chronic exposure of pesticide sprays to pollinators both commercially managed and native. In the meantime, as some pesticides detected in this study are those sprayed during many mosquito control programs (EPA 2013), we call for the creation of bumble bee registry programs. The bumble bee registry can be run in conjuncture with existing state honey bee registries for the notification of mosquito abatement sprays to protect the purchased commercial bumble bee pollinators.

1.4.4 Transferring bees throughout the bloom

All of the pollination units that were placed in the first pickling cucumber field in 2011 were transferred halfway through the season into the second field. The bees originally from field 1 were continually tracked and recorded as a field 1 treatment, and were never counted towards the data for the second field. Interestingly, the units

in field 2 were found to be more productive by means of the foraging and weight tests than those units designated as field 1. These differences could have been detected because of the mid-season move or other unknown differences between the two fields. Further, the transfer study within the watermelon fields in 2012 resulted in no clear pattern of performance from the transferred colonies. Despite the differences detected in the pickling cucumber transfers, we have concluded that although bumble bee units may perform better when left alone, these units can continue to be productive after being transferred.

1.4.5 Confounding factors

Differences between fields were also seen between the different watermelon fields which can potentially dilute meaning from statistical differences seen with colony treatments. In many tests, for watermelon colonies in 2012, colonies from at least one field out performed colonies in field 2. As mentioned above, this field was within a mile of two other watermelon fields that used commercial honey bees for pollination. The bumble bee colonies were subject to much robbing from honey bees at that location which can offer some explanation of statistical differences detected. This especially can hold true as colonies at field 2 generally showed lower levels of productivity, as determined by the many analyses performed.

In 2012, bumble bees were shipped slightly before the peak pickling cucumber bloom in the intended field. Therefore, it was suggested that the bees be given a little extra sustenance to survive the shipment and the few days drought they would experience once placed in the field. Once the bumble bees arrived they were observed

foraging at such a low frequency that statistical testing could not be performed. Further, all but 5 colonies registered their peak weight on 7/9/2012, the day they were first put in the field. This means that most of the colonies were in decline by the time they were supposed to be starting their pollination duties for the crop. The lack of foraging and thus lack of evidence of nearly any weight gain leads us to question whether or not all bumble bee colonies supplied for the pickling cucumber crops in 2012 were viable. Despite the problems with the bumble bee units in 2012, we believe that based on experiments in 2011 bumble bees are not appropriate commercial pollinators for pickling cucumbers in Delaware, though future studies on this matter are warranted.

1.4.6 Overall recommendations

The data from these experiments allow for conclusions to be reached on how growers should use commercial bumble bees throughout the growing season in certain horticultural crops in Delaware. Colonies must always be placed in the shade. Colonies seem to last longer and be more active along the field edge in natural shade but if growers are concerned with bees staying on the crop, commercial companies should supply growers with shade structures in order to lengthen the time in which bumble bees will forage and their overall productivity within the field. When using the shade structures, quads can easily be blown during a strong wind, and they should be strapped down upon placement within the field.

Growers should allow bumble bees at least 30 minutes to settle before opening up each colony for the first time. If they are not settled the grower runs the risk that

bees will charge out of the colony for the first time without an orientation flight. The orientation flight is important as it will help the bee find its way back to the colony. Bumble bees should be placed in the field after the crop has begun to bloom in order to limit the distraction of other blooming plants that could be sought. This is also suggested to prevent robbing behavior seen in honey bees. When small amounts of forage are available, honey bees may find the bumble bee units and rob them. Further, commercial bumble bee units may successfully be transferred from field to field, provided that before each move, growers leave the colony with the one 'entrance only' hole open to allow foragers ample time to return to the colony.

Bumble bees have shown a certain willingness to forage in strawberry and watermelon fields and can be depended on for their pollination. The same cannot be said in a pickling cucumber field. If placed in this crop, bumble bees will tend to find other foraging sources in terms of both pollen and nectar and will not provide the pollination services that growers seek.

Bumble bees will readily accumulate toxins in their wax and their bodies throughout the season. As the foraging activity of bumble bees begins much earlier in the day, they, and other potentially naturally occurring pollinators in the area, are likely to be affected more by the typically recommended (Krupke et al. 2012, Riedl et al. 2006) early morning sprays than honey bees. When housing commercial bumble bees, growers need to be wary of the foraging differences and utilize the entrance reducing tab on the commercial bumble bee colony. This will limit bumble bee exposure to sprays and may increase longevity of purchased bees. Further, given that

some of the chemicals detected during this study are those sprayed for mosquito abatement practices (EPA 2013), we suggest the creation of a registry program for those that purchase and house commercial bumble bees. Such a program could require bumble bee owners to be notified before mosquito abatement insecticides are sprayed. With such notification, growers would then have the ability to close the bumble bee colonies to help prevent any unintended contact of the commercial bumble bees to the mosquito insecticide sprays. Ideally, this program would run in conjuncture with the preexisting registry for honey bees, run through many state agriculture departments, including one in Delaware.

As the species *B. impatiens* occurs naturally in almost all locations where the commercial bumble bees are available, growers should dispose of the bumble bee units as soon as their pollination needs are fulfilled. It is likely that the genetic stock of the commercial bumble bee colonies does not mirror that of the native populations and commercial bumble bees are known to produce more queens than their naturally occurring counterparts and (Ings et al. 2006). As natural genetic diversity is essential in overall population health especially in social insects such as bumble bees (Wilson-Rich et al. 2009), it is important to limit the amount of commercial bumble bee genes from mixing with the naturally occurring populations. Therefore, the mating of the two bee types should be prevented whenever possible. Closing the colony opening tabs or freezing each unit, overnight, are means for colony disposal.

Chapter 2

BEST MANAGEMENT PRACTICES ON USING COMMERCIAL BUMBLE BEES (*BOMBUS IMPATIENS*) ON HORTICULTURAL CROPS IN DELAWARE

2.1 Introduction

As honey bee populations have been declining over the past decade due to numerous pests and pathogens (such as the varroa mite, small hive beetle, amongst others), their ability to over winter has also been declining leaving commercial beekeepers unable to provide commercial pollinators (Artz and Nault 2011, Stanghellini et al. 1997, 1998, Stubbs and Drummond 2001, vanEngelsdorp et al. 2007, 2008) for the agricultural sector. This decrease in managed pollinator populations during the blooming period of many important crops has growers and pollination ecologists concerned and looking to alternative pollinators. To help combat pollinator shortages and meet the pollination requirements of their crops, researchers and growers are investigating the effectiveness of native pollinators in larger agricultural settings. A common native pollinator in Delaware is the Common Eastern Bumble Bee (*Bombus impatiens*). The behavior, physiology and morphology of bumble bees make them ideal pollinators because of the speed at which they transfer pollen, the efficiency with which they gather pollen within various crops, and the increased endurance to fly in adverse weather for longer periods of time (Stubbs and Drummond 2001, Stanghellini et al. 2002, Desjardins and Olivera 2006, Young et

al. 2007). Further, the bumble bee has the ability to buzz pollinate or sonicate the flower for pollen, a pollination technique not seen in honey bees. Buzz pollination occurs by bumble bees vibrating the flower by pumping their wings at a certain frequency, to dislodge pollen from certain sticky anthers (Buchmann 1985, Desjardins and De Oliveira 2006, Goulson 2010, Heinrich 2004**,** King 1993, Kwon and Saeed 2003, Stubbs and Drummond 2001, Velthuis and van Doorn 2006). This is a very efficient method of gathering pollen from poricidal dehiscent crops such as tomatoes, eggplant, and cranberries (Buchmann 1985, Kwon and Saeed 2003, Stubbs and Drummond 2001). Other studies have shown that bumble bee foraging activity starts earlier and ends later in the day than managed honey bees and they forage in lower temperatures (Desjardins and De Oliveira 2006, Goulson 2010, Heinrich 2004). Honey bees begin to forage around 16ºC or 60ºF, whereas bumble bees will start at 10ºC or 50ºF (Goulson 2010, Heinrich 2004, Velthuis and van Doorn 2006).

In the early 1980s, commercial rearing of bumble bees for pollination services was developed in The Netherlands. Within three years 95% of tomato growers in the country were using bumble bees in their greenhouses. By the 1990s commercial bumble bee production made its way to the United States, with the new world species, *B. impatiens* and *B. occidentalis*. Due to drastic declines in native *B. occidentalis* populations, the species is no longer used (Goulson 2010).

Currently, there are two species of bumble bees available globally, *B. terrestris* and *B.impatiens. B. terrestris* is native to Europe and *B. impatiens* is endemic to the eastern United States. In the United States, there are two companies that distribute

commercial nests of *B. impatiens*, Koppert Biological Supply Company and Biobest®. Despite slight differences in nest design, the general concept remains. Bumble bee colonies are reared and placed within plastic boxes, equipped with enough pollen and a sugar substance to satiate bees whilst shipping. A large cotton swab is also placed within the plastic box to allow bees to insulate their colonies. Two holes are engineered into each plastic box that allows the grower to control the activity of their bumble bee nest. These entrance holes are controlled by a plastic flap that either opens or closes all or one entrance hole (Figure 2.1).

Figure 2.1. The colony entrance reducing tab, closed, one open hole, two open holes. The one open hole allows for bees to enter the colony but prevents bees from escaping, a useful tool to keep bees protected from pesticide sprays. Photo credit, J. Marchese

Two open holes indicate the free movement of bees into and out of the colony. Pushing the flap down provides for one open hole and allows only bees to enter as a valve blocks the access of bees to return outside. Each plastic box is placed within a slightly larger cardboard box. Two, three or four cardboard boxes, that include single colonies, are then placed within a corrugated plastic box that protects these units from the elements (Figure 2.2).

Figure 2.2. Pictures of the different components of a commercial bumble bee quad. Top left, inside the individual colony unit. Top right, the whole individual colony unit. Bottom left, inside the quad with three out of the four colony units. Bottom right, the full quad. Photo credit, J. Marchese

Commercial bumble bees are now the leading avenue to pollinate greenhouse tomatoes, worldwide. Countries in which bumble bees are prohibited (due to concern of the establishment of an alien bumble bee species) are unable to compete in the greenhouse tomato market (Goulson 2010). While the necessity of commercial bumble bees has been realized and well-studied in the greenhouse tomato system, little is known about the ability of bumble bees to perform in the open field. Bumble bees differ greatly from their more popular, honey bee, counterpart and thus have different biological requirements in order to optimize their use to growers in the field.

This document will provide Best Management Practices (BMPs) to aid growers in optimizing the use of commercial bumble bee units in horticultural crops, specifically strawberry, watermelon, and pickling cucumber in the state of Delaware.

2.2 General Recommendations

Bumble bees can be used for strawberry and watermelon but not for pickling cucumber horticultural crops. Overall, bumble bees can be used to successfully pollinate strawberry and watermelon horticultural crops during the growing season in Delaware. During our two year study that looked at bumble bees in strawberry and watermelon horticultural crops, bumble bees were constantly and consistently detected in the fields throughout both seasons. Although we determined that strawberry and watermelon pollen were not the main pollen sources being brought into the colonies, foragers were seen with enough frequency and abundance that we are confident in their ability to pollinate these crops. The bumble bees observed on strawberry and watermelon blooms were most likely nectar foragers, but if foragers are strictly on the crops for the intention of gathering nectar, transfer of pollen should still be occurring. The same recommendation cannot be made in pickling cucumbers as bumble bees seem disinterested in foraging in this crop and cannot be relied upon for adequate pollination services. After two years of sampling pickling cucumber fields with commercial bumble bees, they made up at most 8% of all pollinators collected and they were frequently seen on weedy forage such as morning glory, ragweed, horse nettle and other common flowers. Honey bees were the most abundant pollinator found in these fields followed by native sweat bees and pollinating hover flies. Although the results from our study do not favor bumble bees in pickling cucumbers, with all scientific experimentation, further testing is warranted. Until then however, we recommend that commercial bumble bees are not a reliable pollinator for

pickling cucumbers and should not be considered when planning or purchasing pollinators for the crop. Growers should continue to rely upon and use honey bees in pickling cucumber plantings.

Place bumble bees in the field after crops have begun to bloom. When using bumble bees in your crop, growers should follow the typical recommended timing for placement of pollinators within the field. As a rule of thumb, make sure the crop has started to bloom before placing any pollinators in the field (Delaplane and Mayer 2000). Like honey bees, bumble bees need access to forage to sustain themselves. If not enough forage is available they will not be able to nourish their colony or will have to travel further distances in search of pollen and nectar, causing unnecessary strain on their energy resources. Bees that have found unintended forage in the beginning of the season are likely to continue to forage on this unintended source, especially if it is more favorable than the intended crop. Although removing this unintended forage is an option, there is controversy to this method. Many times, areas with unintended forage are at field and forest edges. These edge areas are typically favorable habitats for many native bees that could be providing additional pollination services for your crop (Delaplane and Mayer 2000). Therefore, it is best to wait until your crop first begins to bloom before placing any commercial pollinator in your field and place bees in the middle of the field to encourage in field foraging.

Allow time for bees to settle before opening units. Always follow instructions provided by the bumble bee supplier when placing bees within the field. Give the allotted time before opening up the colonies for the first time. Although

bumble bees will need to chew out of the hole in order to begin foraging, colonies should be given at least 30 minutes to settle after being handled during shipment and placement. If they are placed and opened within the field too quickly, they will have a tendency to rush out of the colony without an orientation flight allowing for workers to lose their way back to the colony and ultimately cause a reduction in colony population numbers. Also, be sure to check on each colony 2-3 hours later to make sure that the bees have successfully chewed out of the hole and exited the nest. On occasion, bees will not successfully chew out of the hole and will need to be cut out of the colony. Although this has been known to occur, it is not common and most colonies will successfully find their way out of their colony and into the crop, on their own. Further, check on bumble bee colonies periodically throughout the bloom to ensure that they are viable and actively foraging. Be sure to notify the supplier of any problems especially if you are concerned with their level of activity.

Close bumble bee units before each pesticide application. During the season, change each bumble bee colony entrance to one open hole at least two hours before all pesticide applications. This will allow time for bumble bee foragers to return and be kept in the colony in order to limit forager exposure to pesticides, see the pesticide section below for more information.

Dispose of bumble bee colonies in a timely and humane fashion. There is a risk of commercial bees breeding with native populations. At a certain point in the season, all bumble bees (wild and commercial) will begin to produce new queens and males. These 'reproductive' bees will eventually leave the nest in search for a mate.

The commercially available bumble bees are the same species as naturally occurring bumble bee species along the entirety of the east coast. This poses a risk that the commercial bees will mate with the naturally occurring, native populations. Commercial bumble bees are mass reared in a factory, and therefore the genetic diversity of the commercial bees does not mirror what is naturally found and occurring in the wild bees. The integrity of this wild genetic stock is important because it allows for the bees to be adapted to a wide variety of environmental conditions and exposure to various pathogens that they may encounter. If commercial bees mate with wild bees, the commercial bees will be diluting the genetic stock of the wild bee population. The commercial bumble bees will begin to produce the reproductives mid to late summer. As the exact timing of each colony's reproductive cycle will vary (Delaplane and Mayer 2000), it is hard to predict the exact time when the reproductives will be released. Therefore, in order to limit the amount of interbreeding between commercial and native bumble bees, immediately after you no longer need the bumble bee pollinators, remove colonies and dispose of them humanely. Commercial colony boxes may be placed in freezers over night as a humane way of disposal. You may also close both holes with the plastic tab to isolate the bees from the environment.

2.3 Colony Placement

Bumble bees can be placed in the middle or on the edge of the field.

Generally bumble bees tend to perform slightly better in the natural shade along the edge of the field. However, as long as bumble bee units are outfitted with some sort of

shade structure; bees will do fine whilst placed in the middle of the crop. Although no significant differences were detected on the collection of unintended pollen from bees placed in the field and those placed on the edge, concerns remain that bees may be more likely to find unintended forage when placed along the field edge. With this in mind, bumble bees can be placed randomly throughout the middle of the field and they will be successful pollinators. If destructive harvest of the crop in question is warranted, bumble bees may be successful pollinators when placed along the field edge.

Place bumble bees under shade, to increase productivity and longevity of the bumble bees. Bumble bee units placed in natural shade (along forest/field edges) or fitted with a shade structure last longer and are significantly more productive than units in the sun. Productive colonies mean that bees are leaving the colony to forage more and bees are bringing in more pollen and nectar into their colony thus increasing the number of pollinators transferring pollen on the crop. Placing bees under shade is especially important during the warm summer months during Delaware's watermelon bloom. Bumble bees constantly and actively strive to keep their colony temperature at around 30^0C (86^0F). They regulate the temperature of their colony by fanning their wings, producing heat, and insulating their colony. As mentioned above, commercial bumble bee colonies are packaged in two layers of cardboard boxes, with another plastic box, and a large cotton ball used for insulation. This nest design although effective in providing a secure location for colony development, provides little ventilation. The temperature of the units can easily reach or exceed the 30^0C

temperature in direct sunlight, regardless of day time temperatures, making shade imperative for optimal pollination production. Figure 2.3 below demonstrates how bumble bees regulate the temperature of their colony, regardless of shade treatment.

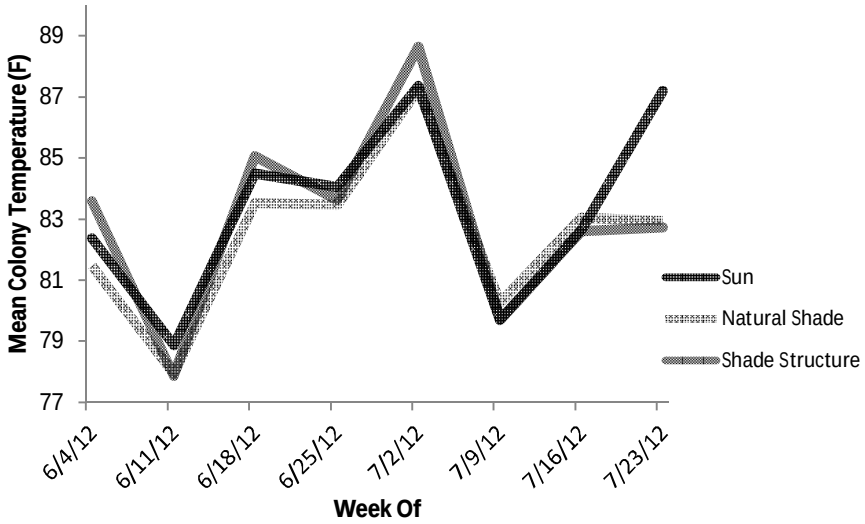

Figure 2.3. Temperatures within colonies of different treatments throughout a growing season.

The colonies exposed to direct sunlight have to work harder and use more energy to thermoregulate and to maintain the optimal 30^0C temperature. At the end of the season, the temperature of the sun treated colonies rises, demonstrating that the sun colonies cannot maintain normal worker activity (pollen and nectar foraging and duties within the colony) for as long as the colonies with shade. When energy that should be available for normal worker activity is allotted to cooling the colony, less energy is available to pollinate the intended crop (Heinrich 2004), decreasing worth to growers. Units placed in the shade will pollinate more and for longer throughout the

season than units in the sun. Although longevity will vary on different colonies and be dependent on other factors such as weather and temperature, generally a grower can expect a commercial colony to be actively pollinating for 6-7 weeks.

Our experimentation also included testing various shade structures currently being developed by the bumble bee supplier. Despite having differences in design, in general, all structures were effective in providing the bumble bees with enough shade to increase productivity. Therefore, any structure that will provide the bumble bees with shade can be effective.

Keep bumble bees away from honey bees. Bumble bees should be placed as far from honey bee hives as possible. This is especially true when crops are not in bloom. When forage is low for the commercial pollinators they should be kept as far away as possible from each other. The current recommendation by Koppert Biological Systems is 100 yards, but honey bees robbing bumble bees has been reported from bees placed much further from each other. Honey bees are very resourceful and a bumble bee colony is a great source of pollen and nectar which honey bees are constantly seeking. If surrounding forage is low or not agreeable to honey bees, bumble bees will be susceptible to honey bee robbing causing a weakened colony and overall loss in productivity from both pollinator species. Despite their smaller size, honey bees can easily take over a bumble bee nest because of their large numbers. Figure 2.4 below is a photograph taken in a pickling cucumber field when bumble bees and honey bees were placed too close together and too early in a field. In

the absence of a suitable foraging source, the honey bees robbed each bumble bee colony in the field.

Figure 2.4. Honey bees robbing out a commercial bumble bee colony. This is a result of placing bumble bees and honey bees too early and too close together in a pickling cucumber field. Photo credit: J Marchese

Strap down bumble bee units. Bumble bee units should be weighed or strapped down, especially when placed within a shade structure. These units may be susceptible to being flipped or carried by strong winds. Not only does this disrupt the normal orientation of the colony, causing helpless larvae, nectar and pollen to fall out of their individual waxen cells, but can cause blockage to the unit openings, trapping bees within the unit. Any such major disruption can have an effect on productivity and should be avoided, whenever possible. This not only occurs during large tropical storms in mid to late summer but can also happen during a typical summer thunderstorm. Figure 2.5 below is a photograph of a quad that was found flipped over after a thunder storm in a 2012 watermelon field.

Figure 2.5. Bumble bee quad, flipped over after storm. Photo credit: J. Marchese

Bumble bee units may successfully be transferred to another field.

Bumble bees may be transferred to another field for additional pollination services throughout a season. Before moving, close the plastic opening tab to the one-hole open position. Allow forager bees at least two hours to return to the colony. The bumble bee colony may then be transferred to another site. Keep in mind that the longer the plastic opening tab is held at the one-hole position, the more foragers will be able to return before the colony is moved. Bumble bee units that are moved have had mixed results on season long productivity, but will still forage at a new site.

2.4 Pesticide Sprays

Close up colonies before each spray. Bumble bees very easily accumulate pesticides within the wax of their brood clump and their bodies by foraging in crops that have been treated with various chemistries. Currently there is literature on physical signs and symptoms of bees (honey bees and bumble bees) after pesticide poisoning (Riedl et al. 2006). Although physical effects of poisonings are important to

note, the actual toxicity level will help to determine safe levels of pesticide application. Unfortunately, little work has been done to test the actual toxicity levels of the different chemicals in bumble bees. Although studies have been done to test toxicity of pesticides on honey bees, the varying biologies of the two insects do not allow for a direct translation on the bumble bee. Further, much of the information available on toxicity is for an acute exposure of a few days, to one pesticide, at most. Pollinators within the field can chronically be exposed to multiple pesticides, especially when fields are sprayed at weekly intervals. Recently, there has been evidence that honey bees exposed to a combination of multiple pesticides causes a synergistic effect causing more harm to the bee. Thus little is known on how toxicity of chemicals, sprayed consistently throughout the season, will affect bumble bees. Bumble bees are known to start foraging earlier in the morning than honey bees, thus early morning sprays that are suggested in order to protect honey bees, would not reduce exposure of bumble bees (and many other native, naturally occurring bees in the area). Although bumble bees inevitably will always have some exposure to sprayed pesticides within the field, growers can limit exposure by using the plastic opening tab within each colony box. Growers are urged to close up the commercial nests at least two hours before spraying to decrease the exposure of bees to the pesticides. This is done by switching the plastic opening tab to only one-hole opening. Bees will be able to fly into the colony, but will be not able to get out, limiting direct exposure to sprayed pesticides, and perhaps increasing productivity and overall longevity of the pollinators purchased for the field.

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Appendix

PERMISSION LETTER

Jacquelyn Marchese <marchese@udel.edu> Fri, Apr 19, 2013 at 9:34 AM

To: "Baris.Reuben@epamail.epa.gov" <Baris.Reuben@epamail.epa.gov>

Hi Reuben,

I am Deborah Delaney's graduate student at the University of Delaware. We spoke briefly around the holidays about my M.S. thesis on using commercial bumble bees for crop pollination. Specifically, the exposure of these bees to pesticide applications in the field. In my analysis of this topic for my thesis, I used two tables published in an EPA document. Despite citing the EPA as the source of these tables, I was informed that I needed written permission to use this information before submitting my thesis to the University for graduation. The tables are on page 159 in the "White Paper in Support of the Proposed Risk Assessment Process for Bees" (document is attached to this email). Can you advise on how this can be done?

Any insight that you can give would be appreciated.

Thank you,
Jacquelyn Marchese
--
Jacquelyn Marchese
M.S. Student
Department of Entomology and Wildlife Ecology
University of Delaware
marchese@udel.edu

EPA White Paper in Support of the Proposed Risk Assessment Process for Bees 2012.pdf
2442K

Baris, Reuben <Baris.Reuben@epa.gov> Fri, Apr 19, 2013 at 9:48 AM

To: Jacquelyn Marchese <marchese@udel.edu>

Jacquelyn,

I will look into this and get back to you shortly. As far as the underlying data, it is not owned by the Agency, it was compiled from and EFSA (European Food Safety Authority) lit review. But I will get back to you shortly on the written permissions you requested.

Hopefully I'll have something by COB today, if not then early next week.

Best,

Reuben

Baris, Reuben <Baris.Reuben@epa.gov> Fri, Apr 19, 2013 at 3:48 PM
To: Jacquelyn Marchese <marchese@udel.edu>

Jacquelyn,

Just so I'm clear, the University of Delaware requires that you have written permission? The document you cite is a public document…just wanted to make sure I knew who to ask on this topic.

reuben

From: Jacquelyn Marchese [mailto:marchese@udel.edu]
Sent: Friday, April 19, 2013 4:11 PM

[Quoted text hidden]
[Quoted text hidden]

Jacquelyn Marchese <marchese@udel.edu> Thu, Apr 25, 2013 at 10:51 AM
To: "Baris, Reuben" <Baris.Reuben@epa.gov>

Hi Reuben,

Just checking in to see if you were successful in getting a permission letter to use the tables, previously discussed, in my thesis. Let me know if you need any additional information.

Thanks,
Jackie Marchese
[Quoted text hidden]

Baris, Reuben <Baris.Reuben@epa.gov> Thu, Apr 25, 2013 at 10:55 AM
To: Jacquelyn Marchese <marchese@udel.edu>

Hi Jackie,

I'm still working on it. I'm not sure who it should come from, and no one is giving me a straight answer except that since it's a public document why is UD requiring permission to use the information?

I'll hopefully have something for you today.

reuben