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Trapping and Control of the Small Hive Beetle, Aethina tumida, an Invasive Parasite of Honey Bees, Apis mellifera

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TRAPPING AND CONTROL OF THE SMALL HIVE BEETLE, *AETHINA TUMIDA*, AN INVASIVE PARASITE OF HONEY BEES, *APIS MELLIFERA*

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by
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Accepted by:
Dr. William M. Hood, Committee Chair
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Dr. William C. Bridges

ABSTRACT

The small hive beetle, *Aethina tumida* Murray (Coleoptera: Nitidulidae), is one of the most recent honey bee pests of economic importance, especially in the southeastern United States. Various in-hive traps have been developed to attempt to control the populations of this invasive pest within honey bee colonies. The first year of my research focused on comparing the effectiveness of three commercially available traps for removing small hive beetles. Thirty-two colonies were established with 0.9-kg package bees with a queen in four apiaries. Eight colonies were placed in each apiary, each colony randomly receiving one of four treatments: the three-chambered Hood trap, the disposable Better Beetle Blaster, the Freeman tray trap, or having no trap as a control colony. Two of each type of colony treatment was present in each apiary. Data was collected over a seven month period from April to November 2010. The Freeman tray trap was determined to be the most effective at capturing small hive beetles compared to the other traps and the controls. More adult beetles were consistently trapped within the Freeman trap over the season, with the Better Beetle Blaster and Hood trap capturing more beetles in late summer than earlier in the year.

The first and second years of my research additionally investigated the possibility of adult small hive beetles being attracted to dead beetles within traps for a "trapping sink" effect. Five apiaries of three colonies were established with 0.9-kg package bees in 2010 and fifteen apiaries of three colonies were established with 0.9-kg package bees in 2011. The "sink" colonies in each apiary were treated with both the Better Beetle Blaster and Freeman tray trap. In 2010, two apiaries were treated with one sink, two apiaries were treated with two sinks, and the remaining colony had no traps as a control. In 2011, the same treatments were used in the same ratio with six one sink apiaries, six two sink apiaries, and three control apiaries. The data for both investigative years was collected from April to November and compiled for a total of 20 apiaries and 60 colonies. The lack of significance between the trapped and control apiaries demonstrated that there is no discernible “trapping sink” effect when a percentage of colonies within an apiary have small hive beetle traps. This suggests that the recommendation for small hive beetle control within an apiary would be to place traps in every colony.

DEDICATION

I dedicate this culmination of my academic endeavors and research investigations to my family for all their support and love for the past two years of challenge and change.

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CHAPTER ONE

LITERATURE REVIEW

Introduction

Overview

The small hive beetle (SHB), *Aethina tumida* Murray, has become a serious problem to beekeepers in the United States in the last decade. The SHB is originally a native of Africa and was described by Murray in 1867 (Neumann and Elzen 2004). In June of 1998, the SHB was first identified in St. Lucie, Florida after being previously collected, but unidentified, in 1996 and 1997 in Charleston, South Carolina (Hood 2004). Due the coastal nature of both cities, as well as the fact that both possess large ports, the SHB may have found its way to the United States via cargo ships from its homeland (Hood 2004). The SHB has been shown to cause significant damage to honey bee colonies that are weak or under great stress, but also effects strong bee colonies to the point of absconding (Ellis 2004). One way in which the beetle may adversely affect the colony is through the spoilage of stored honey and pollen, most likely through beetle defecation. This leads to honey that is unusable to both the bees and the beekeepers (Hood 2004). The detrimental effect of this pest on colonies and the economic impact on the beekeeping industry has prompted research into the biology and control of the small hive beetle in recent years. Attractants have been tested to determine their attractiveness to the beetles (Nolan IV and Hood 2008b). From this research, various traps have been created to aid in the control of this honey bee pest. The effectiveness of each trap must be determined in order for a control method to be produced that will benefit the beekeepers in lowering beetle populations in colonies. This information will also provide both small-scale and commercial beekeepers with the knowledge to make an informed decision on the most cost effective method of control for a particular operation size.

Economic Impact

The SHB is not a major pest in its homeland of Africa, since it has less economic importance where beekeeping is less commercial. The SHB is mainly a threat to weakened or stressed colonies of honey bees. However, the SHB has become a major pest for commercial beekeepers in the southeastern United States due to their ability to infest even strong colonies of European honey bees (Hood 2004). Many beekeepers reported massive colony losses that were attributed to the SHB after its identification in the United States in 1998 (Hood 2004). Afterwards, a quarantine was placed on Florida to try to minimize the spread of the pest, but it was lifted after the SHB was discovered to have crossed the quarantine line, making its elimination impossible. Not only has the SHB spread from Africa to the United States, but has also found its way to Australia (Hood 2004, Neumann *et al*. 2010), Egypt (Mostafa and Williams 2002), and even into Canada (Dixon and Lafreniere 2002). DNA variation in the haplotype of SHB found in the United States suggests that the beetle may have been introduced multiple times into North America instead of one introduction that has since spread (Evans *et al*. 2003)

Healthy colonies of honey bees can be susceptible to invasion and even collapse if the SHB numbers become too great for the colony to manage naturally (Hood 2004). After infestation, colonies can increase in tendency to leave the hive entirely, or abscond (Elzen *et al*. 1999). However, the African honey bee, *Apis mellifera scutellata*, has a greater tendency towards this behavior than the more commercialized European honey bee. The SHB can cause much greater damage to European colonies than African or Cape bees, *Apis mellifera capensis* (Ellis *et al*. 2003c). In its native homeland of Africa, the SHB is classified as a scavenger due to the absconding nature of the African honey bee. When the bees simply leave the hive due to the infestation, even with minimal damage, the SHB consume the leftover hive products as scavengers (Lundie 1940, Schmolke 1974).

Even though the SHB may not be a major pest of the African honey bee because of the increased tendency of the bees to abscond, it has become a major pest of the European honey bee, which is more prevalent in commercial beekeeping. The European honey bee has become the most significant source of pollination for some domestic crops in the United States. The SHB infestation in the late 1990's caused numerous reports from beekeepers on colony losses numbering in the thousands in the United States (Somerville 2003). The progression of SHB invasion begins with the adult beetles entering the hive, which slowly leads to a build-up of beetles. If unhindered by the bees, the female SHB then begin to reproduce and the resulting larvae cause severe damage to the brood, honey, and pollen. When the larvae are mature, they leave the hive in mass numbers and pupate in the soil, after which they reemerge and infest the same colony or a different colony. The honey bees may be able to use some behaviors to naturally defend against the invasion, but a weakened colony or one with a high SHB infestation may not be able to withstand the multiple generations of intruders.

Small hive beetles damage colonies through the consumption of honey, pollen, and bee brood. Female adult beetles can lay eggs in sealed brood by perforating the capping or wall of the comb. This damages the comb and also leaves the bee brood vulnerable to consumption by the beetle larvae (Ellis and Delaplane 2008). The beetles also spoil stored honey through defecation (Lundie 1940, Schmolke 1974). The feces of the beetles and larvae increases the moisture content of the honey from its normal 18% (Graham *et al*. 1999) to an unstable amount that causes the honey to ferment (Lundie 1940). This honey cannot be consumed by humans or the honey bees, and can lead to colony mortality through starvation. In 1998, United States beekeepers lost an estimated $3 million due to SHB damage to honey supers (Elzen *et al*. 2001). The SHB may also be a potential vector for honey bee viruses such as deformed wing virus (Eyers *et al.* 2009a) and sacbrood virus (Eyer *et al*. 2009b), leading to increased risk of colony death and lower productivity in infected colonies. The queen and package bee business has also been negatively affected by the SHB invasion, due to concern by beekeepers over the spread of the beetles in queen cages and bee packages. Reports of beetle eggs possibly being attached to honey bees suggested that even transportation of queens was risky. Importing fruit with SHB on it was also a possible means of spreading the pest. These factors led to a ban on all packages of bees from the United States to the United Kingdom (Brown *et al*. 2002).

The SHB will also feed and reproduce on several varieties of fruit, such as bananas, mango, grapes, and strawberries (Buchholz *et al.* 2008), as well as avocado, cantaloupe, pineapple, honeydew, and starfruit (Eischen 1999). When given the choice, it is clear that their preferred food sources are bee brood, honey, and pollen. However, when there are no honey bees available to provide this food source, the SHB can survive on fruit products (Buchholz *et al.* 2008). Nevertheless, the fruit industry should have minimal risk of transporting and spreading SHB due to the lower affinity the SHB have for fruit.

The infestation of native bumble bee colonies has also been studied, where the SHB have been able to live and reproduce under controlled conditions (Spiewok and Neumann 2006). Beetles were shown to be attracted to pollen and to bee colonies, so the beetles may be able to find hosts through odors produced by bee products within the colony. Bumble bees may be able to serve as alternative hosts for the SHB and need to be protected as much as the European honey bee, since they are native pollinators in the United States (Spiewok and Neumann 2006). More research must be done into the interactions between SHB and bumble bees to determine the effects of this pest on the native bees.

Biology of the Small Hive Beetle

The SHB is a member of the insect family Nitidulidae of the order Coleoptera. The Nitidulidae family is distinguished from other similar beetles by transverse procoxal cavities, grooved metacoxae, dilated tarsal segments, small forth tarsi and three-segmented antennal club (Neumann and Elzen 2004). The adult beetles vary in size depending on food resources and the environment, but generally average 5.7 millimeters in length and 3.2 millimeters in width (Ellis *et al*. 2002c). Female adult beetles have been estimated to lay 1000 to 2000 eggs in their lifetime after reaching sexual maturity

(Somerville 2003). Female SHB generally oviposit into pollen or brood comb, but have been observed to chew through capped brood and oviposit on the bee pupa (Ellis 2004). The larvae of the SHB mature within the hive and exit the colony in order to enter the surrounding soil to pupate.

Small hive beetle eggs are generally laid in the cracks and crevices of the beehive to prevent removal by honey bees. The eggs are laid in clusters and are white in color, with an average length of 1.4 millimeters and an average width of 0.26 millimeters. The eggs normally hatch within 3-6 days and the timing is dependent upon the humidity (Hood 2004) and temperature (Guzman and Frake 2007). The larvae reach an average length of 1.0 centimeter when fully mature. The larvae are cream-colored and mature on bee brood, honey, and pollen over an average period of 13.3 days inside the honey bee colony and finish maturation in 3 days in the soil before pupation. The pupation stage lasts an average of 8 days, with females pupating slightly faster than males (Ellis 2004). In this stage, soil moisture plays a key role in the development of the pupae, as dry soil impedes the ability of the pupae to reach adulthood. However, the soil type does not inhibit the growth of the pupae, and as long as the soil is moist, beetles will become a pest problem in any soil (Guzman *et al*. 2009). The lifespan of an adult SHB is dependent upon food sources available. With an ideal diet of pollen and honey, the adult beetles have been observed to live upwards of 6 months, but may live longer when overwintering in the hive (Lundie 1940).

The SHB also may oviposit and reproduce on fruit when honey bee brood, honey, and pollen sources are unavailable. SHB have been observed to survive on various fruit diets (Buchholz *et al.* 2008, Eischen 1999). However, the success of reproduction on such diets is lower and beetles will only use fruit as a viable food source if there are no available bee colonies (Buchholz *et al*. 2008, Ellis *et al*. 2002a). Even though the SHB can survive on a diet of fruit, it reproduces and thrives on a diet of pollen, honey, and bee brood (Ellis 2003b, Neumann *et al.* 2001a). The SHB can be classified as a scavenger that also is a natural pest to honey bee colonies, due to its ability to survive on fruit, and as a facultative parasite because of its preference for products from bee colonies.

The odors produced by the honey bee colony have been thought to attract SHB (Torto *et al*. 2005 & Torto *et al*. 2007). Bioassays found adult SHB to be attracted to volatiles from honey bees, fresh pollen, and unripe honey (Torto *et al*. 2005 & Torto *et al*. 2007). The yeast *Kodamaea ohmeri* has been found in colonies with high SHB infestations (Benda *et al*. 2008) and the volatiles from this compound are also attractive to SHB (Torto *et al*. 2007). The SHB may also be attracted to unidentified pheromones of other SHB in the hive, but this idea needs further inquiry as to whether this theory could offer control methods for SHB.

Control Methods

Behavioral

Honey bees also have natural methods of controlling the SHB invaders in the colony. Honey bees can detect abnormal brood and remove it to defend against pathogens and parasites (Ellis *et al.* 2004a). It has been previously observed that honey bees remove small hive beetle eggs and larvae in both the European honey bee, *Apis mellifera*, and the Cape honey bee, *Apis mellifera capensis* (Ellis *et al.* 2004a). When the SHB oviposit their eggs into any cracks or crevices in the hive that can be found, the bees will actively remove the eggs that can be reached. The bees also appear able to detect infested sealed brood and remove the eggs laid within the cells (Guzman 2008). The missed eggs hatch into larvae, which can be removed by the bees to allow for natural beetle control in the hive in African colonies (Neumann and Hartel 2004). However, in European colonies, beetle larvae numbers may be too great for the bees to naturally control and will eventually overrun the colony. This happens especially in colonies stressed or weakened by disease, parasites, or too many adult SHB. These problems will distract the worker bees away from the normal hygienic behavior and cause the hive to be overrun by the larvae (Ellis *et al.* 2004a).

Honey bees will also contain adult SHB in “prisons” that are often made of propolis and the bees will forcefully confine beetles into these spaces. They may also encapsulate the beetles in spaces using propolis, possibly due to the difficulty in piercing the exoskeleton of the beetles (Neumann *et al.* 2001b). It has been observed that bees will imprison several beetles and will push them back into the prison if they attempt to escape (Ellis 2005). The beetles survive in this state by attempting to induce their guards to regurgitate honey. This is done by antennal contact that often causes an aggressive response in the guard bees. However, if the beetles persist, they can induce a trophallatic response in the honey bee (Ellis 2005). This behavioral mimicry seems to not only be beneficial to the beetle, but is also helpful to the bees. The more trophallatic behavior that occurs, the easier it is to confine the SHB for the guard bees due to the lack of motivation for the beetles to escape. However, in the European honey bee, an increase in beetle numbers can affect this balance. The more SHB there are, the less beetles are being fed by their captors, which may allow them to escape back into the comb where they are harder to guard. The increasing beetle population also increases the number of guard bees needed, which strains the colony since less workers are foraging and more are on guard duty (Ellis 2003b, Ellis *et al*. 2003d).

The different races of honey bees have slight differences in their hygienic behavior towards the SHB. The African honey bee, *Apis mellifera scutella*, can deal with high infestation levels as it carries out the removal of the eggs and larvae from the hive (Neumann and Hartel 2004). The African honey bee also uses more propolis in constructing the beetle prison than its European relative, which seems to not only improve confinement of the beetle, but also limits the spaces available for the SHB to oviposit eggs, limiting the number the bees have to guard (Neumann and Hartel 2004). When the European honey bee was compared to the Cape honey bee for effectiveness in removing beetle eggs and larvae, both races demonstrated preferential removal of SHB infested brood (Ellis *et al.* 2004a). Both the African and Cape honey bees also seems to be able to confine and defend against SHB even at high infestation levels (Ellis 2005). It is possible that certain genetic strains of the European honey bee may be able to better confine and hygienically respond to the SHB invasion, but more research and breeding must be done to integrate that trait into modern beekeeping.

Biological

Biological controls from natural pathogens and fungi may offer methods of controlling SHB populations. Several fungal pathogens have been investigated due to beetle mortality (Ellis *et al*. 2004b). The fungal pathogens *Aspergillus flavus* and *Aspergillus niger* were discovered on dead beetle larvae and are known for attacking other insects that burrow into the soil (Hood 2004). Studies involving these pathogens took adult beetles that had been killed by the fungal agents and managed to extract and culture more of the pathogens. SHB larvae were then inoculated with the fungal pathogens and the adult beetle emergence was compared to control beetles with no fungal exposure. It was concluded that there was no significant beetle mortality for the *A. niger* treatments. However, the beetles infected with *A. flavus* showed a 38% mortality rate, which differed from the 3% for the control group (Richards 2005). However, there are several problems with the *A. flavus* fungal pathogen. It is known to produce a potent aflatoxin that can cause aflatoxicosis and liver cancer in humans, and using this species as a control measure may increase the risk of honey contamination. Another concern is that *A. flavus* may cause 'stonebrood' in honey bees, which could become a major problem with a concentrated use of this pathogen (Richards 2005).

Three different strains of the bacterium *Bacillus thuringiensis* (*Bt*) were tested for effectiveness in mortality of SHB. The bacterium produces crystals that become toxic upon digestion by the insect. The concentration was placed on the strains to inhibit reproduction of the SHB due to the destructive nature of the larvae. However, all three strains were found to have no significant effect on the number of larvae produced (Buchholz 2006). Further research must be done to determine the potential control properties of other *Bt* strains or other bacterium.

Another biological control method may be found in soil-infesting entomopathogenic nematodes, which seem to infect SHB at the prepupal stage. The nematodes *Heterorhabditis megidis* (‘HO strain) and *Steinernema carocapsae* (‘All strain) were tested, but further research must still be done to determine the control that may be offered by this method (Cabanillas and Elzen 2006).

Chemical

There have been chemical products that have been developed for controlling SHB populations, but results indicate that this is not the most efficient form of control. Lundie originally suggested the use of carbon disulfide to fumigate stored comb (Lundie 1940). Normal household bleach has also been recommended for killing both adult beetles and their larvae inside of honey houses (Park 2002). Comparisons of effectiveness between pyrethroids, botanical extracts, and organophosphates against different life stages of SHB demonstrate that there is varying vulnerability by the larvae and adults to different chemical treatments (Ellis and Delaplane 2007).

Only one product has been developed for treating SHB inside beehives. The product is the organophosphorous compound coumaphos, which is marketed under the product name CheckMite+ (a.i. 10% coumophos plastic strip, Bayer Corp, Shawnee Mission, Kansas, 66201, USA). This treatment comes in the form of plastic strips that are placed at the bottom of the hive. When the beetles hide at the bottom of the hive, they come into contact with the strips and expire (Hood 2004). This product was first tested by Elzen (1999) as a viable and effective treatment option for SHB infestation. However, CheckMite+ is very toxic to both humans and honey bees, making it dangerous to use commercially around honey supers.

A second chemical product has been developed for use outside the hive. GardStar (a.i. 40% permethrin, Y-Tex Corp, Cody, Wyoming, 82414, USA) is a synthetic pesticide that is extremely toxic to the honey bees. The product is mixed with water and applied to the soil around the colonies in order to kill any beetle larvae that intend to pupate in the ground (Hood 2004).

Chemical treatments have had varying degrees of success, but the potential for the beetles to build tolerance to the chemicals with increased usage has led to research into other ways to control the population of SHB within a honey bee colony.

Cultural

There are a few key ways in which beekeepers may limit the expansion of a potential SHB problem. Reducing the stress on a colony and providing maintenance that promotes strong colonies is necessary. A stronger colony will not be as predisposed to have a SHB problem as a stressed or weakened one. Especially in regions where the SHB is a particular problem, maintaining well-populated colonies through good beekeeping practices will reduce the risk of infestation. Management that limits mite problems, brood disease, wax moth activity, swarming, queen failure, and starvation all lower the stress on the hive (Hood 2004).When feeding colonies with sugar water or sugar patties, great care and maintenance is necessary as the beetles are also attracted by sugar (Westervelt *et al.* 2001). It is also known that the SHB larvae will pupate in moist soil, so keeping colonies in sunny, open, and dry areas away from irrigation may limit beetle reproduction (Ellis 2004).

Sanitation in the honey house and around the hives themselves may also limit beetle activity. Cleaning the honey house and proper storage and maintenance will limit the attractants and food sources available to the adult SHB. Honey supers should have the honey extracted within 2-3 days to prevent damage to the honey and the comb. Wax and pollen stores should be secured to prevent SHB from finding and reproducing in them. Keeping the honey house dry and at a relative humidity below 50% will help to desiccate SHB eggs (Somerville 2003). Freezing infested comb taken from the colony will kill all the life stages of this pest. Bleach can also be used to clean the honey house and infested comb, since it is an effective and rapid method of killing SHB (Park 2002).

Trapping

Several different traps have been developed to control the SHB numbers without harming the honey bees or contaminating the honey. Earlier developed traps have attempted to exclude beetles from entering the hive using in-hive traps or smaller entrances (Hood 2004, Elzen *et al*. 1999). A study by Smolke (1974) conducted several simple experiments with three in-hive traps, two of which were designed for a position above the inner cover and the last being placed at the back of the colony on the bottom board. Due to the cold weather and bee inactivity, these investigations proved inconclusive.

Other means of controlling SHB invasions have been in the modification of the entrance into the colony. A study by Ellis *et al*. (2002b) replaced the traditional

Langstroth entrance with ¾ inch sections of PVC pipe approximately 3-4 inches above the bottom board. The conclusions of the study found that the modification to a single PVC pipe can reduce colony invasion by allowing the bees to guard a smaller space. However, the reduction of the entrance size led to reduced brood production, impaired thermoregulation of the hive, and poor water drainage, which negatively effected the colony. The reduced hive entrance control method was further evaluated by Hood and Miller (2005) for a much longer period of time. There was no overall effect on the SHB population due to the reduced upper hive entrance, but the reduction in bee brood was noticeable. The reduced entrance was not recommended as a control method due to the problems with bee productivity resulting from lower brood numbers (Hood and Miller 2005). Another study by Ellis *et al.* (2003a) used screen bottoms to try to mitigate the problems outlined in the previous study. Two types of polyvinyl chloride (PVC) pipes were used, with diameters of 1.9 centimeters and 3.8 centimeters, which were compared to the normal open entrances. The varying results proved inconclusive, and the suggestion was made that factors such as apiary location and nectar flow could have affected colony strength and therefore the level of SHB invasion. There was again an issue with reduced brood production with the smaller entrances, which was only slightly aided by the addition of the bottom screen.

Elzen *et al*. (1999) developed a SHB trap using a plastic bucket. The trap was designed with pieces of 8-mesh hardware cloth glued to holes with 7.0 centimeter diameters in the bucket. The openings were large enough for the beetles, but too small to allow any bees through. The buckets were randomly placed throughout apiaries with known infestations of SHB and checked at 24 and 48 hour intervals. The traps were baited with various hive product combinations, and the most attractive combination being the honey, pollen, and live honey bee mixture. However, these traps were limited in their control application, possibly due to competing odors from surrounding colonies.

A study by Torto *et al*. (2007) used two types of in-hive traps. One trap was a modified Langstroth bottom with a rectangular opening that was 18x14-cm in its center. The hole was covered with four-mesh aluminum screening to prevent the bees from entering the trap. The modified bottom board was then attached to a three-sided frame, with the missing side positioned toward the back or side of the hive, with runners to allow the trap to slide into position. Below the hole, the lid of an egg container was placed and held the bait and two openings in the middle were fitted with polymerase chain reaction (PCR) plates, and the tray was painted black since beetles prefer the dark. The traps that were baited with an inoculated pollen dough trapped significantly more beetles than unbaited traps, showing the importance of attractants in controlling SHB. Levot (2008) has developed a refuge trap that utilizes a corrugated cardboard insert treated with fipronil and encased in plastic. An estimated 62% SHB mortality was detected within 6 weeks of treatment application.

Other in-hive traps have been developed with different levels of effectiveness. One of these traps is the Hood beetle trap (Brushy Mountain Bee Farm, Moravian Falls, North Carolina). This trap is currently on the market and was developed by Dr. Michael Hood, Clemson University (Nolan & Hood 2008a). The trap is a three-chambered plastic box that can be fastened to the bottom bar of a frame and placed in the bee colony in place of a normal frame in either a honey super or brood chamber with relatively little difference in the number of SHB trapped (Nolan & Hood 2008a). The top of the trap has slits that allow the beetles to enter, but excludes bees from entering. The middle chamber is filled with an attractant, which is normally cider vinegar and the two outward compartments are filled with mineral oil (Hood 2004). The beetles end up falling into the oil, which coats their bodies and appendages, preventing escape and the beetles eventually die. However, this trap is merely meant to reduce a SHB infestation and cannot eliminate the pest completely (Nolan & Hood 2008a).

Another in-hive trap that is currently available is the Cutts Trap, which is better known as the Better Beetle Blaster (Dadant & Sons, Hamilton, Illinois, United States). The Cutts trap is a disposable plastic trap that has square openings in the top to allow for beetles to enter the trap and prevent bee entry. The trap is thin and placed in between frame top bars, with the top resting on the top of two adjacent frames. The trap is half-filled with vegetable oil, which has a similar effect to the mineral oil in the Hood trap.

A third available trap is known as the Freeman trap (Ashley Bee Supply, Hamburg, Arkansas, United States). This trap consists of a specially designed screened bottom that allows a plastic tray to slide into it and under the colony. The screen attempts to prevent bees from being trapped, while allowing beetles to enter. The tray is partially filled with vegetable oil to drown beetles that run to the bottom of the hive to escape harassment by honey bees. This trap must also be restored at least biweekly due to its tendency to accumulate debris from the colony. However, this trap allows the beekeeper to monitor the SHB in a colony with limited stress on the bees, since the trap can be accessed without opening or excessively disturbing the colony.

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CHAPTER TWO

COMPARISON OF THREE TRAPS TO CONTROL THE SMALL HIVE BEETLE, *AETHINA TUMIDA*, IN HONEY BEE COLONIES

Summary

To compare the effectiveness of three commercially available in-hive traps for controlling the small hive beetle, *Aethina tumida* Murray, within European honey bee, *Apis mellifera* Linnaeus, colonies, the number of beetles trapped was monitored biweekly from 19 May to 2 November 2010. Four apiaries were established with eight colonies in each apiary, for a total of 32 colonies. The Freeman, Hood, and Better Beetle Blaster traps were each randomly placed in two beehives out of the eight present in each apiary in April 2010. The remaining two colonies were control hives having no traps. Colony strength was monitored by measuring the amount of capped bee brood, adult bees, and honey every eight weeks. Varroa mite numbers were monitored using detector boards to determine if the small hive beetle traps were having an effect on the varroa mite population. All test colonies were dismantled at the end of the experiment to determine the number of small hive beetle adults remaining following the 7-month trapping period. Colony measurements and varroa mite numbers showed no significant differences over the course of the experimental period. There was a significant increase in the mean number of small hive beetles in Freeman traps during the experimental period overall and on 19 May, 1 June, 11 June, 23 July and 18 October individual sampling periods. There was no significant difference in the number of small hive beetle remaining behind in the colonies at the end of the 7-month period. The data as a whole suggest that the Freeman trap is the most effective trap for controlling small hive beetles.

Keywords: Small Hive Beetle, *Aethina tumida*, *Apis mellifera*, traps, pest control, Freeman trap, Hood trap, Better Beetle Blaster

Introduction

After its initial collections in 1996 and 1997 in Charleston, South Carolina, the small hive beetle (SHB), *Aethina tumida* Murray, was positively identified in St. Lucie, Florida in June of 1998 (Hood 2004). Since then, this native pest from Africa has spread throughout the United States and has become a serious concern for southeastern commercial and small-scale beekeepers. The small hive beetle (SHB) causes significant damage to European honey bee colonies through fermentation of the honey stores, destruction of comb, and a general decrease in the overall efficiency of the colony (Hood 2004). This damage can often result in the loss of even a strong colony of bees (Hood 2004), which not only impacts the finances of the beekeeper, but also reduces the population of the world's most important pollinator. To control the SHB, various methods have been developed including chemical, cultural, mechanical, and physical means. However, these methods do not guarantee SHB control. Various SHB traps have been developed, but few investigations have been conducted to compare their effectiveness.

Two chemical treatments have been developed in the United States to control SHB. One currently on the market is CheckMite+ ® (a.i. 10% coumophos plastic strip, Bayer Corp, Shawnee Mission, Kansas, 66201, United States). This plastic strip is stapled to a small piece of corrugated cardboard that is placed on the bottom board of the hive, with the treatment underneath the cardboard facing down (Elzen et al. 1999). When the beetles attempt to hide from bees and light, they come into contact with the pesticide and die. A second available treatment is GardStar ® (a.i. 40% permethrin, Y-Tex Corp, Cody, Wyoming, 82414, United States). This product is registered as a ground drench to treat the soil around the hive and kill the SHB larvae attempting to pupate. However, improper use of both products may lead to honey contamination and to chemical resistance by the beetles. Therefore, alternative methods of control must be developed for this pest.

A previously studied means of controlling this pest was the use of a smaller hive entrance where the guard bees can more effectively reduce the infestation of the colony by the SHB. Although a modification of the hive entrance reduced the invasion of the colony by the SHB, it also led to a decrease in bee brood production, impaired thermoregulation of the hive, and caused poor water drainage, which negatively affected the colony (Ellis et al. 2002). Further evaluation of this method over longer periods of time produced similar results in significantly reducing the brood production in the colony (Hood and Miller 2005).

Various possible substances have been tested to determine their attractiveness to the beetles (Nolan and Hood 2008). A trap developed by Elzen et al. (1999) for use outside of the colony used a bucket loaded with hive products and 8-mesh hardware cloth glued across holes 7 cm in diameter in a bucket to allow small hive beetle entry. Traps for use inside the colony were developed by Torto et al. (2007) and used the beetle’s affinity for darkness by painting the modified bottoms black. However, both studies produced inconclusive results with limited effectiveness, prompting further research into other trapping methods.

One of the more environmentally friendly traps is the Hood beetle trap. This trap is currently on the market and was developed at Clemson University (Nolan & Hood 2008). The trap is a three-chambered plastic box with the middle chamber filled with an attractant, apple cider vinegar, and the two outer compartments are half-filled with mineral oil as a killing agent (Hood 2004). The beetles enter the one-way beetle trap and are incapable of exiting the trap due to the oily residue on their bodies.

Another in-hive trap that is currently available is the Cutts trap, which is better known as the Better Beetle Blaster. The Cutts trap is a disposable plastic trap that has small square openings in the top to prevent bee entry but allow beetles to enter the trap, which is half-filled with vegetable oil. A third available trap is the Freeman trap. This trap has a specially designed screened hive bottom that allows a plastic tray to slide into it from the rear and under the hive. The tray is partially filled with vegetable oil, which acts as the lethal agent for beetles as they enter the trap to evade bees.

The current study compares the effectiveness of the Hood trap, Freeman trap, and Cutts trap based on the number of SHB captured over a 7-month period and the number of beetles remaining in the colonies at the conclusion of the experiment. Colony estimates of the brood, adult bees, and honey were taken to determine colony strength and productivity. Varroa mite numbers were sampled to determine when the colonies needed to be treated for mites. It is hypothesized that the Freeman trap will be the most effective method of control based upon adult beetle numbers trapped in preliminary investigations (Hood and Tate 2010).

Materials and Methods

On 6 April 2010, 32 colonies of honey bees were established in the Clemson University Experimental Forest in Pickens County, South Carolina, United States. Apiaries were established with 0.9-kg packages of bees and mated queens purchased from Wilbanks Apiaries, Claxton, Georgia, United States. Each colony was housed in a 10-frame Langstroth beehive with a honey super. Additional honey supers were added as needed. The packaged bees likely contained similar incipient SHB populations upon delivery and the test colonies were likely invaded during this investigation by immigrating beetles from surrounding areas, which had a proven history of SHB activity. All colonies were placed with similar conditions of partial sunlight and shade. Four apiaries were used with eight colonies in each apiary.

On 3 May, two test colonies from each of the four apiaries were randomly selected to receive one of three trap treatments: Freeman trap, Cutts trap, and Hood trap. The remaining two colonies in each apiary were selected as non-treated control colonies having no traps. The Freeman trap (Ashley Bee Supply, Hamburg, Arkansas, United States) was half-filled with vegetable oil (ConAgra Foods Inc., Omaha, Nebraska, United States). The Cutts trap (Dadant & Sons, Hamilton, Illinois, United States) was half-filled with vegetable oil and randomly placed between frames 1 and 2 or between frames 9 and 10 in the top super. The Hood trap (Brushy Mountain Bee Farm Inc., Moravian Falls, North Carolina, United States) was mounted in a shallow super frame and randomly placed between frames 1 and 2 or between frames 9 and 10 in the top super. The middle compartment of the Hood trap was filled with apple cider vinegar (White House ®, National Fruit Product Co., INC. Winchester, United States) and the two outer compartments were half-filled with food grade mineral oil (Mineral Oil, U.S.P., packaged by: Cumberland Swan Smyrna, Tennessee, United States).

Beginning 3 May, all traps were serviced at approximately 2-week intervals on 19 May, 1 June, 11 June, 28 June, 12 July, 23 July, 10 August, 23 August, 3 September, 20 September, 5 October, 18 October, and 2 November. The trapped beetles were counted and removed and the traps were emptied of and subsequently refilled with oil or vinegar. At 6-week intervals on 15 June, 27 July, 8 September, and 19 October, all colonies were loaded with a Freeman trap tray half-filled with fresh vegetable oil for a 24-hour colony beetle count as determined by the number of beetles removed during a 24-hours trapping period. The 24-hour beetle survey was preceded by a 3-day varroa mite detector board (Dadant & Sons Inc., Hamilton, Illinois, United States) placed in the Freeman trap tray to compare the varroa mite population in each test colony. At 8-week intervals on 22 April, 28 June, 10 August, and 4 October, all colonies were sampled to estimate the following parameters: adult bees in the colony, capped brood, and honey. These parameters were determined by estimating the percentage of each frame covered by of adult bees, capped brood and honey in intervals of 0.1. These were counted by two separate estimators, after which the totals were averaged and recorded for a practical estimate of colony strength based on these colony measurements (Skinner et al. 2001).

On 2 November, total adult beetle populations in all colonies were determined following queen removal and safe-keeping. All hive frames, boxes, bottoms, and tops were removed and shaken on a 2.13m x 1m white plastic table to remove bees and beetles. Beetles were counted and subsequently killed by smashing them with a hive tool. This procedure provided an estimate of beetles remaining in test colonies (Spiewok et al. 2007) following a complete season of trapping, since only a small percentage of beetles may be able to fly or crawl away unnoticed. However, the same method being used at each colony ensures that the estimate is an accurate reflection of the number of adult small hive beetles in the colony.

The data were analyzed by a randomized complete block design analysis of variance (ANOVA), recognizing trap treatment as main effects and apiary locations as block effects, with colonies as sub-samples. Means were separated with least significant differences (LSD) test and Fisher's test, with differences accepted at $P < 0.05$. Analyses were performed for each individual sampling period and totals. All analyses were conducted using the software package SAS (SAS Institute 1992).

Results

There was no significant difference within the samples (all $P>0.05$) in the varroa mite counts or colony strength measurements overall, with capped brood, honey, and adult bee determinations remaining similar throughout the sampling period. On individual sampling periods for the 2-week traps surveys there were significant differences (all $P<0.05$) between the Freeman trap counts and the other two traps on 19 May, 1 June, 11 June, 23 July, and 18 October (Figure 2.1). There were no significant differences (all $P>0.05$) overall or among individual sampling periods in the SHB captured during the 24-hour Freeman trap capture at 6-week sampling periods (Figure 2.2). The least squares mean for the total number of SHB remaining in each surviving colony during the end of project shakeout on 8 November 2010 was not found to be statistically significant (P=0.0707) (Figure 2.3). The least squares mean of the total number of SHB captured was significantly different (P=0.0335) over the 7-month sampling period for the colonies treated with the Freeman trap (Figure 2.4).

Discussion

Over the 7-month experimental period from May to November of 2010, the Freeman trap was significantly more efficient at trapping adult SHB within colonies of European honey bees. Not only did the Freeman trap capture significantly (P=0.0018) more SHB overall (Figure 2.4), but it also trapped significantly more SHB on several individual sampling periods (all P<0.05) during the biweekly SHB counts (Figure 2.1). Position difference in beehives between the bottom-hive Freeman trap and the other two top-hive traps is unlikely to account for the discrepancy in the number of SHB trapped (Nolan and Hood 2010). Our research determined that the Freeman trap is the most effective method of removing the SHB from a colony during the year when compared to the Hood and Cutts traps.

The Hood and Cutts traps captured significantly fewer SHB overall during the 7-month experimental period. However, the biweekly sampling periods indicate an increased number of SHB trapped in the Hood trap from mid-summer to late fall, with the peak captures occurring on the 23 August and 5 October sampling dates (Figure 2.1). The number of SHB trapped in the Hood trap decreased in both the 2-week and 6-week sampling periods after 5 October (Figures 2.1 and 2.2). This decrease might be linked to a lower number of beetles in the colonies since new generations are not emerging in the colder months and the remaining adults are expected to cluster with the bees (Ellis et al 2003).

The number of SHB trapped in the Cutts trap remained relatively constant throughout the 2-week sampling periods (Figure 2.1), except 28 June and 12 July, when the beetles are in greater numbers due to an accumulation of generations (Guzman et al. 2010). The consistency of the numbers might be attributed to the lack of an attractant and the beetles find the trap in summer and fall at the same rate in the hive. Our research suggests that even though the Freeman trap is the most effective method for removal of adult SHB, the addition of either the Hood trap or the Cutts trap placed in the top of the same hive should maximize the number of SHB captured and removed from a colony. The number of SHB trapped during the end-of-experiment shakeout was not statistically significant (P=0.0707) overall in the trapped colonies and the control colonies. However, the p-value for the significance of the Freeman trap versus the control during the shakeout was rather close at 0.0707. Even though that is not a statistically significant value, this value is significant for a beekeeper in a logical sense. The low value indicates some relative difference between the Freeman trap and the control. For a beekeeper, even the smallest difference may aid in controlling the SHB, so even the statistically insignificant values are valuable in reality.

Eight of the colonies were lost over the course of the experimental period. Two colonies of each treatment type (Freeman, Hood, Cutts, and control) were lost during the year for various reasons that did not appear to be attributed to SHB. The survival of these colonies may have either decreased or increased the perceived efficiency of each trap.

However, even without having the numbers from those colonies, the Freeman trap is clearly the most effective, because it captured significantly more SHB overall (Figure 2.4).

The SHB counts remained relatively consistent in each of the four apiaries throughout the year, with a seasonal decrease in the number of SHB trapped corresponding to an equal decrease in all apiaries. However, the location of each apiary was taken into consideration as a possible source of variability in beetle numbers and the strength of the colonies. Similar sun and shade was provided for all colonies, with any stress by management of the colonies being similar for each colony. Colony strength based on the adult bees, brood, and honey showed no significant differences overall ($P>0.05$) and the similarities in location conditions suggest limited effect on SHB populations in each apiary.

Even though there is a statistically insignificant difference between the Freeman trap and the control colonies during the shakeout, data gathered in several individual sampling periods are highly suggestive of the effectiveness of the Freeman trap. The total SHB trapped in the Freeman trap was significantly different ($P=0.03$) from the other traps (Figure 2.4), and several individual 2-week sampling periods had significant differences in the SHB captured with the Freeman trap (Figure 2.1). The conclusion that can be gathered from multiple data sources is highly suggestive that the Freeman trap is the most effective trap out of the three compared traps for controlling the SHB. However, control of this pest inside honey bee colonies might be maximized with the Freeman trap when used in combination with other traps, such as the Hood or Cutts traps, since these traps did capture a number of SHB later in the year (Figure 2.1).

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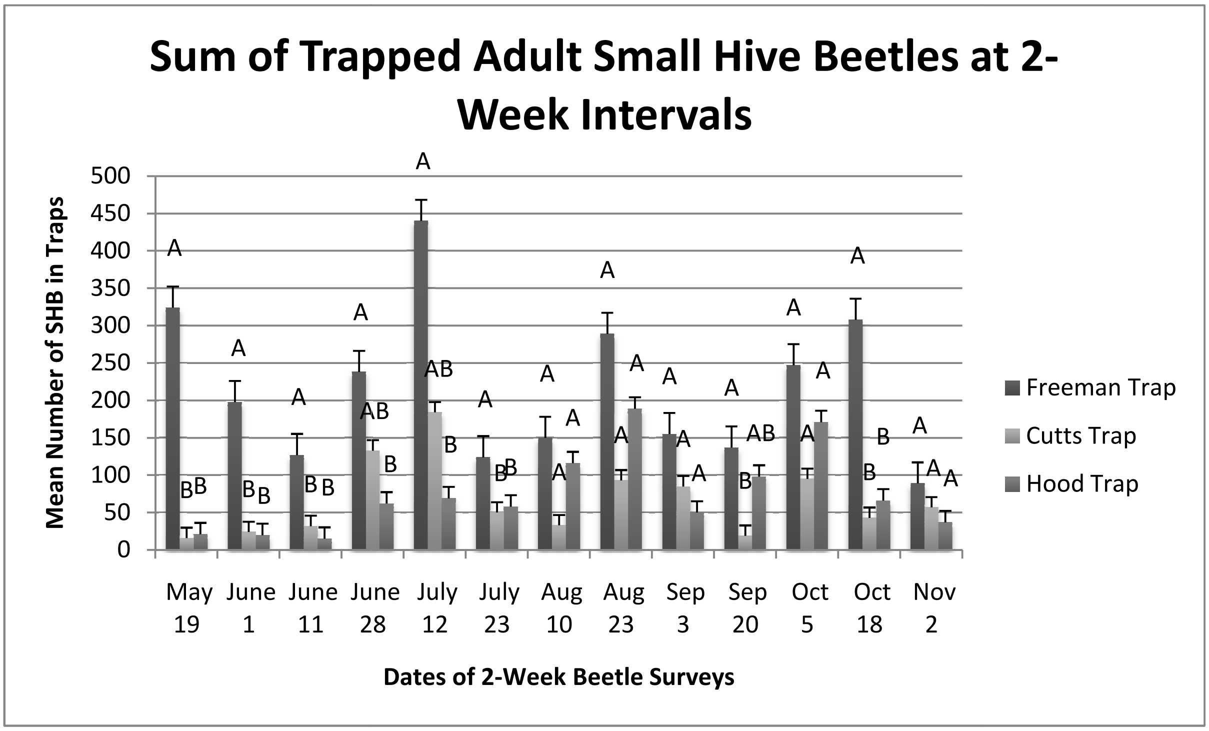

Figure 2.1: Mean number of adult SHB + standard error from variation captured in the three small hive beetle traps during 13 sampling periods in Pickens County, South Carolina, May-November 2010. Different letters indicate significant differences (P<0.05).

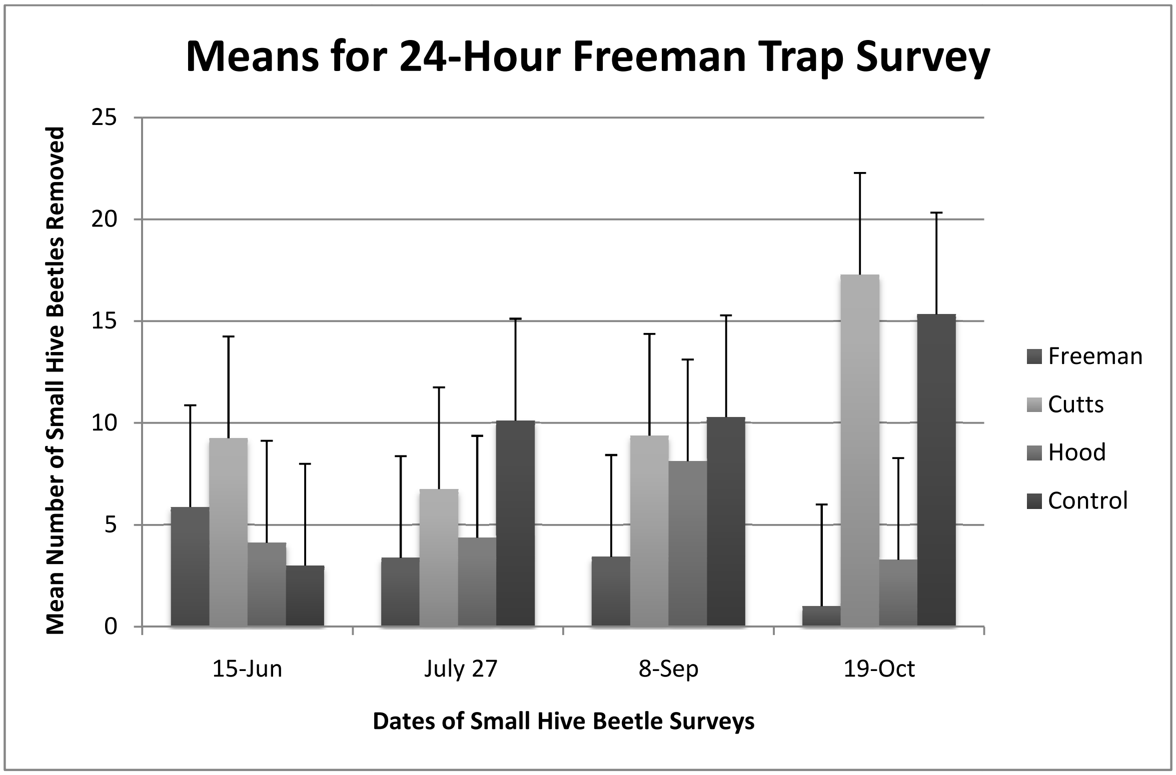

Figure 2.2: Mean number of adult SHB ± standard error from variation caught in Freeman traps during four 24-hour sampling periods at 6-week intervals for all colonies in Pickens County, South Carolina, May-November 2010.

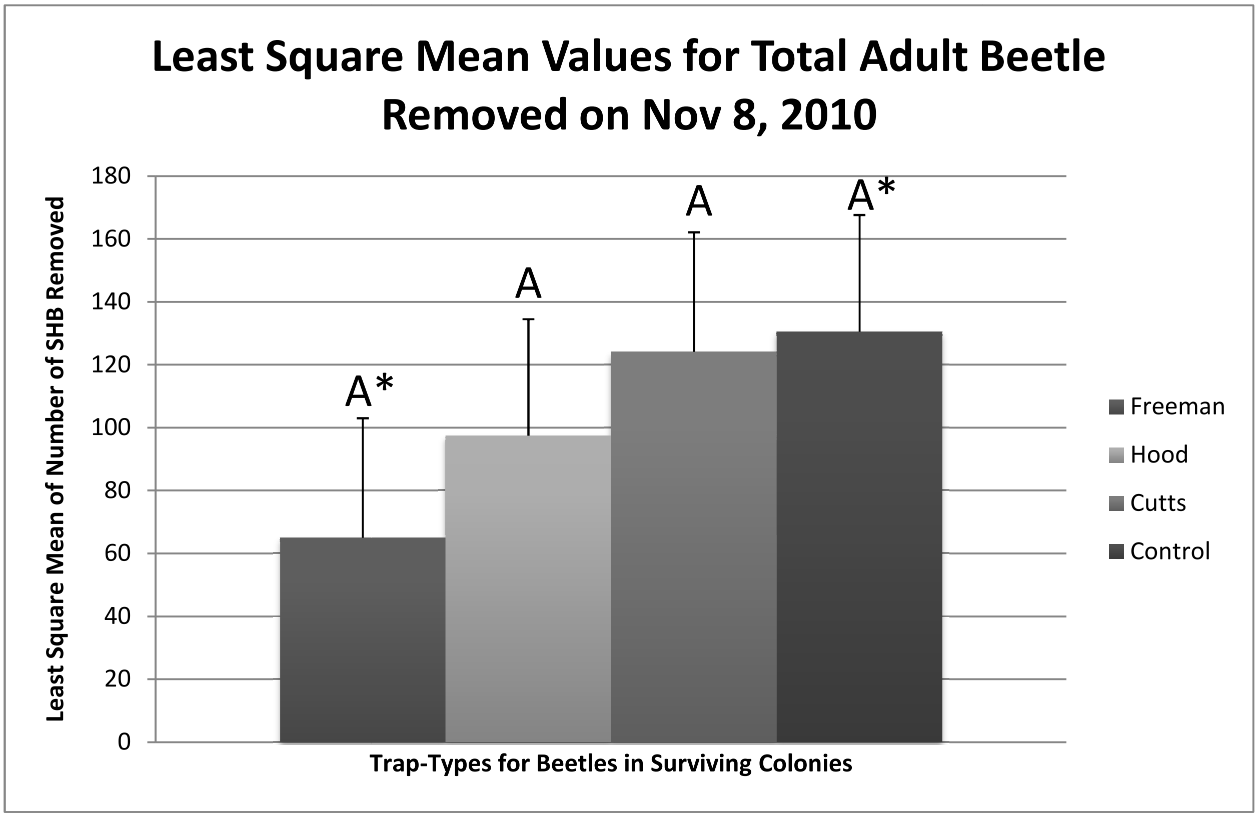

Figure 2.3: Least square mean analysis of the total number of adult SHB shaken out of and killed with a hive tool from colonies ± standard error from variation comparing each separate trap-type and control following the 7-month trapping period in Pickens County, South Carolina, May-November 2010. No statistically significant differences were found overall over the experimental period ($P>0.05$). However, the statistical difference (labeled as A*) between the Freeman trap and the control ($P=0.07$) is highly suggestive of an increase in the effectiveness of the Freeman trap in capturing SHB inside a colony.

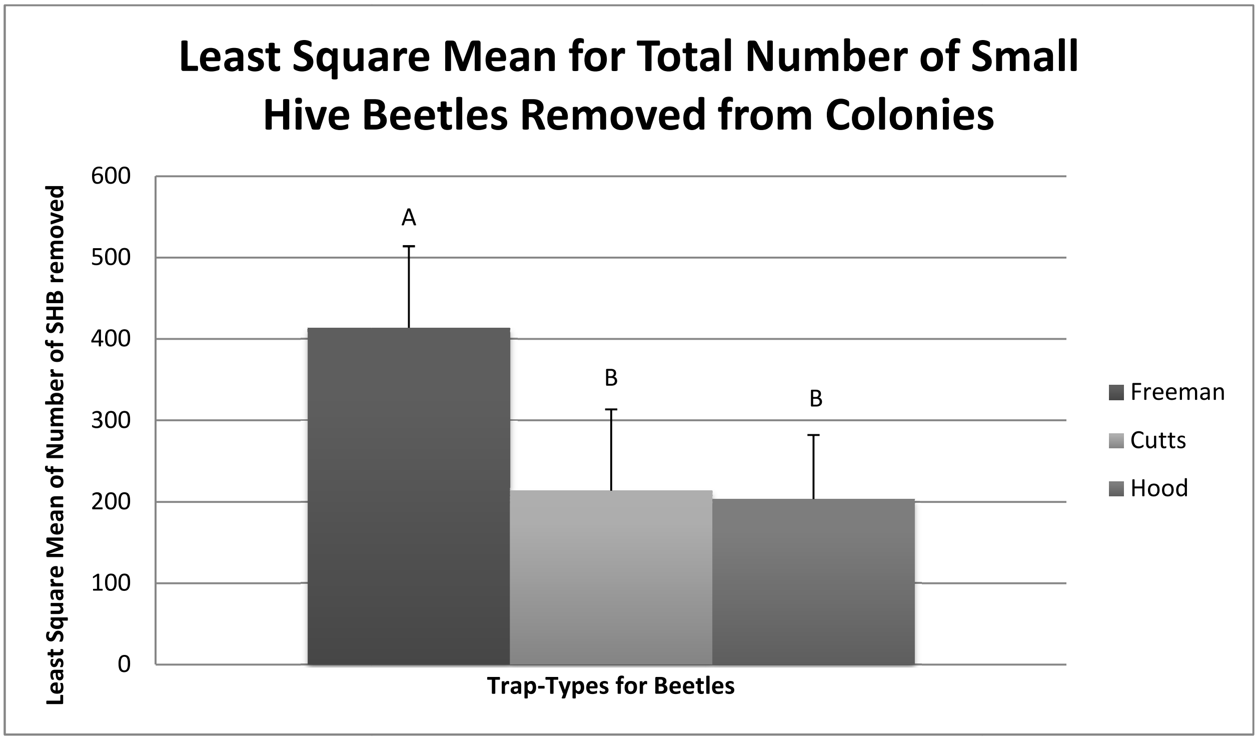

Figure 2.4: Least square mean analysis of the total number of adult SHB removed from colonies ± standard error from variation utilizing each trapping method over the 7-month trapping period in Pickens County, South Carolina, May-November 2010. Different letters indicate significant differences (P<0.05).

CHAPTER THREE

INVESTIGATION INTO "TRAPPING SINKS" TO CONTROL SMALL HIVE BEETLES, *AETHINA TUMIDA*, IN APIARIES OF HONEY BEES, *APIS MELLIFERA*

Summary

Comparing several traps for their effectiveness at capturing small hive beetle adults led to the observation of lower numbers of beetles being trapped in control colonies than expected. This observation led to the development of the "trapping sink" theory that small hive beetles accumulating in traps of treated colonies could be attracting other beetles, which would decrease the number of beetles in non-trapped control colonies within the same apiary. To test this hypothesis, investigations were performed in 2010 and 2011 from April through November for both years. Fifteen colonies were established with 0.9kg package bees in 2010 in five apiaries of three colonies and an additional 45 colonies were established with 0.9kg package bees in 2011 in 15 apiaries of three colonies. In the year 2010, two apiaries had traps in one colony to act as a sink, two separate apiaries with two colonies with traps to act as sinks, and one remaining apiary with no traps as a control. The year 2011 had six apiaries with one colony as a sink trap, another six apiaries with two sink trap colonies, and three control apiaries with no traps. Colonies were established in the Clemson University Forest and were isolated by a distance of at least 0.4km. Small hive beetles captured were counted every 2 weeks, and traps were emptied and restored. Surveys of adult small hive beetle numbers were conducted in every colony in a 24-hour trap survey every 6 weeks following a 3-day varroa mite survey. Colony strength measurements were monitored by estimating percent of frame coverage of capped brood, honey, and adult bees every 8 weeks. In November of each investigative year, each colony had all bees and beetles “shaken out” and all the adult beetles were counted. The results from both years were combined to give a dataset of 60 colonies to investigate the “trapping sink” theory. The end-of-project shakeout of every colony showed no significant difference in the number of beetles remaining in each apiary treatment of either one sink trap apiary, two sink trap apiary, or no sink trap apiary. There was no significant difference found in the colony measurements between the treated and non-treated apiaries or by date. However, there was a significant difference when mite counts were compared between the three apiary treatments. The 2-week surveys showed a significant difference between the apiaries with one trapped colonies and the apiaries with two trapped colonies on one date comparison, but there was no significant difference overall. The 24-hour surveys showed a significant difference in the number of small hive beetles trapped at one date comparison, but there was no significance difference overall between the three apiary treatments. Results indicate that no significant effect occurs when traps are placed in only a few colonies in an apiary. Therefore, the current recommendation would be to control adult small hive beetle population by placing traps in every colony.

Keywords: *Apis mellifera, Aethina tumida,* honey bee, trapping sink, small hive beetle, apiary control

Introduction

The small hive beetle, *Aethina tumida* Murray, is one of the most recent invasive pests of European Honey Bees, *Apis mellifera* Linneaus, to enter the United States and has become a serious issue for beekeepers. Since the small hive beetle’s (SHB) initial discovery in South Carolina in 1996 and its subsequent identification in Florida in 1998 (Hood 2004), various methods have been developed including chemical, cultural, mechanical, and physical means to control its population. A smaller hive entrance has been investigated as a possible method to reduce invasion of SHB by allowing the bees to guard a smaller entrance. Ellis et al. (2002) reduced the entrance of the colonies by replacing the traditional hive entrances with PVC pipe sections. The entrance modifications did reduce the number of SHB, but also decreased the brood production, impaired the bee's ability to thermoregulate the colony, and caused poor water drainage (Ellis et al. 2002, Hood & Miller 2005). Traps have been developed and investigated in order to attempt to control this honey bee pest without using chemicals, contaminating the honey, or harming to the bees.

A trap developed for use outside the colony was developed by Elzen *et al*. (1999), which used a bucket loaded with hive products and 8-mesh hardware cloth glued across holes 7-cm in diameter in the bucket to allow SHB entry. However, these bucket traps were found to be limited in their use in controlling the SHB, which may have been due to overpowering attractant odors from the surrounding bee colonies. A study by Schmolke (1974) conducted several simple experiments with three traps for use inside the hive, two of which were designed for a position above the inner cover and the last being placed at the back of the colony on the bottom board. Due to the cold weather and bee inactivity, these investigations proved inconclusive. A study by Torto *et al*. (2007) used two types of in-hive traps. One trap was a modified bottom board with an opening covered by mesh to exclude adult bees. A container with bait was placed beneath the hole to attract adult beetles, and the container was painted black to exploit the SHB affinity for darkness.

Several in-hive traps have been more recently developed and investigated for their effectiveness in removing adult SHB from colonies of honey bees. These traps aid in reducing the populations of SHB within colonies, but do not eliminate them completely. One of these traps is the Hood beetle trap (Brushy Mountain Bee Farm, Moravian Falls, North Carolina). The trap is a plastic box with three chambers that is attached to a single hive frame and placed in the bee colony in place of a normal honey super or brood chamber frame. Investigations concluded that the placement of the trap in the top super or the brood chamber yielded similar numbers of SHB captured (Nolan & Hood 2008). The top of the trap has longitudinal openings that allow the beetles to enter, but excludes bees from entering. The middle chamber is filled with an attractant, and the two outward compartments are filled with mineral oil (Hood 2004). The beetles end up falling into the oil, which coats their bodies and prevents escape and the beetles eventually die.

Another in-hive trap is the Better Beetle Blaster (Dadant & Sons, Hamilton, Illinois, United States), which can also be referred to as the Cutts Trap, because it was developed by Lawrence Cutts (retired Apiary Chief Inspector, Florida Department of Agriculture, Gainesville, Florida). The Cutts trap is a disposable plastic trap that has square openings 3-mm wide in the top to prevent bee entry and allow for beetles to enter the trap. The trap is sized to be able to be placed in between frame top bars, with the top resting on the top of adjacent frames. The trap is half-filled with vegetable oil, which has a similar effect as the mineral oil in the Hood trap. A third available trap is known as the

Freeman tray trap (Ashley Bee Supply, Hamburg, Arkansas, United States). This trap consists of a modified screened hive bottom that allows a plastic tray to slide into it and under the colony. The screen attempts to prevent bees from entering the trap, while allowing beetles to enter. The tray is partially filled with vegetable oil to drown beetles that run to the bottom of the hive to escape harassment by honey bees.

In 2010, the Hood, Cutts, and Freeman traps were investigated for their effectiveness at capturing SHB inside honey bee colonies. The Freeman trap was found to be the most effective trap for controlling the SHB (Hood & Tate 2010). However, there were far fewer adult beetles found in the control colonies than expected. These observations indicate that the colonies having traps may have provided “trapping sinks” in test apiaries where the dead and captured beetles within the traps may have been attracting more adult beetles to trapped colonies, which would reduce the overall beetle numbers in the control colonies. Therefore, the current investigation into this theory compares apiaries with different numbers of colonies treated with traps, as well as apiaries with no traps as controls. It is hypothesized that the number of beetles captured in the treated apiaries will not be significantly different and that the control apiaries will have a significantly greater number of SHB overall.

Materials and Methods

These investigations were carried out over an experimental period of 2 years from April through November for each year. On 6 April 2010, 15 colonies of honey bees were established in the Clemson University Experimental Forest in Pickens County, South Carolina, United States. Apiaries of three colonies each were established with 0.9-kg packages of bees and mated queens purchased from Wilbanks Apiaries, Claxton, Georgia, United States. On 4 April 2011, 45 colonies of honey bees were established in the Clemson University Experimental Forest in the same general area used for the investigation in the previous year. The apiaries of three colonies each were established with 0.9-kg packages of bees and mated queens purchased from Wilbanks Apiaries. All colonies were housed in a 10-frame Langstroth beehive with a honey super. Additional honey supers were added as needed. The packaged bees likely contained similar incipient SHB populations upon delivery and the test colonies were likely invaded during this investigation by immigrating beetles from surrounding areas, which had a proven history of SHB activity. All colonies were placed with similar conditions of partial sunlight and shade. There were five apiaries used in 2010 and 15 apiaries established in 2011, for a total of 20 apiaries.

On 3 May 2010, two of the first year apiaries were randomly selected to have one colony treated with both a Freeman tray trap on the bottom and a Better Beetle Blaster (Cutts) trap in the top honey super as a “sink”. Two separate apiaries were randomly selected to have two of the three colonies treated with both the Freeman tray trap and the Cutts trap as “sink” colonies. The last apiary was left with no colonies having traps as a control apiary. On 29 April 2011, six of the second year apiaries out of the 15 were randomly selected to have one colony treated with both the Freeman tray trap and the Better Beetle Blaster (Cutts) trap. Six separate apiaries were randomly selected to have two of the three colonies treated with both the Freeman tray trap and the Cutts trap. The remaining three apiaries had no traps in the colonies to serve as controls. The placement of traps within each treatment apiary was also randomized. The Freeman trap tray was half-filled with vegetable oil. The Cutts trap was half-filled with vegetable oil and randomly placed between frames 1 and 2 or between frames 9 and 10 in the top super.

Beginning in April, all traps were serviced at approximately 2-week intervals to count and remove trapped beetles and to refill traps with oil. In 2010, these trap services took place on 23 May, 8 June, 18 June, 6 July, 20 July, 31 July, 16 August, 31 August, 13 September, 28 September, 12 October, 22 October, and 9 November. In 2011, these services occurred on 17 May, 31 May, 10 June, 30 June, 12 July, 22 July, 5 August, 19 August, 1 September, 15 September, 29 September, 13 October, and 1 November. At approximately 6-week intervals on 21 June, 2 August, 14 September, and 26 October in 2010 and 10 June, 22 July, 2 September, and 18 October in 2011, all colonies, including the controls, were loaded with a Freeman trap tray half-filled with vegetable oil for a colony beetle count as determined by the number of beetles removed following a 24-hour trapping period. After the 24-hour survey, the beetles were counted, removed, and the trapped colonies within the trapped apiaries had the tray traps restored. The 24-hour beetle survey was preceded by a 3-day varroa mite detector board (Dadant & Sons Inc., Hamilton, Illinois, United States) placed in an empty Freeman trap tray to compare the varroa mite population in each test colony. The mite numbers for the 3-day detection were averaged to determine the number of mites dropped per day. At approximately 8-week intervals on 7 July, 16 August, and 12 October in 2010 and 30 June, 25 August, and 18 October in 2011, all colonies were sampled to estimate the following parameters: adult bees in the colony, capped brood, and honey. These parameters were determined by estimating the percentage of each frame of adult bees, capped brood and honey in intervals of 0.1. These were counted by two separate estimators, after which the totals were averaged and recorded for a practical estimate of colony strength based on these colony measurements (Skinner et al. 2001).

On 17 November in 2010 and 8 November in 2011, total adult beetle populations in all colonies were determined following queen removal and safe-keeping. All hive frames, boxes, bottoms, and tops were removed and shaken on a 2.13m x 1m white plastic table to remove bees and beetles. Beetles were counted and then subsequently killed by crushing them with a hive tool. This procedure provided an estimate of beetles remaining in test colonies (Spiewok et al. 2007) following a complete season of trapping, since a small percentage of beetles may be able to fly or crawl out of reach unnoticed. However, the same method being used at each colony ensures that the estimate is an accurate reflection of the number of adult SHB in the colony.

The recorded measurements and data for the 2 years were compiled into one dataset of 60 colonies in 20 apiaries. The data were analyzed by a randomized complete block design analysis of variance (ANOVA), recognizing trap treatment as main effects and apiary locations as block effects, with colonies as sub-samples. Means were separated with least significant differences (LSD) test and Fisher’s test, with differences accepted at $P < 0.05$. Analyses were performed for each individual sampling period and totals. All analyses were conducted using the software package SAS (SAS Institute 1992).

Results

The compiled data from both years indicated a significant difference (P=0.0491) was found in the least squared mean between the one trap and two trap treatments for the 2 week captures on the data point labeled d10, which corresponds to data from 28 September 2010 and 15 September 2011. However, comparisons of every other corresponding set of dates showed no significant difference (all P>0.05) for the two apiary treatment types (Figure 3.1). There was a significant difference (P<0.05) at one data point labeled d3, which corresponds to data from 14 September 2010 and 2 September 2011, for the compiled least squared mean number of SHB captured during the 24-hour survey performed every 6 weeks for the three apiary types (Figure 3.2). The compiled least squared mean for the total number of SHB remaining in each colony during the end of project shakeout performed on 8 November, 2010 and 17 November, 2011 was found to have no significant differences (P=0.5353) when comparing the three apiary types (Figure 3.3). There was no significant difference at the apiary level (all P>0.05) in the colony strength measurements, with capped brood, honey, and adult bee determinations remaining similar throughout the sampling period. A significant difference (P=0.0487) occurred in the varroa mite counts when the three apiary treatments were compared between the no-trap control apiaries and the one sink colony apiaries and two sink colonies apiaries (Figure 3.4).

Discussion

The biweekly trap surveys conducted from early May to early November in 2010 and from late April to early November in 2011 showed one significant difference

(P=0.0491) in the number of captured SHB between the apiaries with one sink colony and the apiaries with two sink colonies. This difference corresponds to compiled numbers from mid-September in both years. However, every other point comparison between the one and two sink apiaries showed no significant difference (all P>0.05) (Figure 3.1). The significance at data point d10 seems to be due to an apiary in which the SHB count was extraordinarily high during one sampling performed in September. Therefore, this one point is not representative of the inclination of the other data points, which are not significantly different (all P>0.05) (Figure 3.1). The biweekly trap survey trend appears to support the hypothesis of the "trapping sink" theory. The one sink colony apiaries captured similar numbers of beetles as the two sink colony apiaries, indicating that treating one colony in three will remove the same number of beetles as two colony treatments out of three. This decreases the number of treatments necessary, and therefore also decreases the expense and labor for the beekeeper. However, the results from the 6-week adult beetle numbers and the end of project shakeout beetle numbers contradict this assertion.

The apiary comparison for the number of SHB captured in the 24-hour surveys every 6 weeks showed a significant difference (P<0.05) between the three apiary treatment types at d3 (Figure 3.2). However, there was no significance between the one sink and control apiaries, which contradicts the "trapping sink" theory expectation that the controls would continuously have significantly greater numbers of SHB than the sink apiaries. There is also no significance between the apiary treatment types at any other sampling period. This lack of significance appears to be due to the variation between colonies and between apiaries in colony strength in terms of numbers of adult bees and production of honey. The colony strength is a factor in the population of SHB within the colony since weaker colonies are less able to defend against an infestation (Hood 2004). Therefore, the colony strength variation contributed to the large standard error present in the number of SHB found in the colonies, which in turn caused the SHB counts for each apiary treatment type to be within that error and no significance was found. The number of beetles captured in the control apiaries is also much lower than was expected in both investigation years, which may have contributed to the lack of significance when compared to the treated apiaries in the majority of sampling periods. When the sink apiaries are compared to the control apiaries, a significant increase in the number of beetles in the control colonies would be expected. No significance between the one sink and two sink apiaries would then indicate a similar ability to capture SHB even if there is only one sink present in the apiary. The lack of significance between the apiary treatment types does not support the "trapping sink" theory (Figure 3.2), as well as the one significance for the two colony trap apiaries. The depressed number of adult SHB captured in the two sink colony apiaries suggests that having two sink colonies compared to one sink colony in an apiary of three colonies will lower the population of beetles in the apiary significantly. This does not support the "trapping sink" theory where it would be expected that one sink colony would be able to lower the population of beetles in the apiary significantly more by attracting the beetles to the sink colony.

The number of adult SHB captured during the project shakeout for the 2 years was shown to have no significant differences (P=0.5353) between the three apiary treatment types (Figure 3.3). As previously shown in the 6-week surveys, this lack of significance is possibly due to the amount of variation between colonies and apiaries. The control apiaries also had a much lower number of beetles remaining in the colonies than was expected at the end of the project as reported in previous investigations (Hood & Tate 2010). This is particularly puzzling, since these colonies did not have beetles removed from traps every 2 weeks, since a higher number of beetles would be expected. It is possible that the amount of precipitation in the investigative years was not adequate for keeping the soil moist enough for successful beetle pupation (Linvill n.d.), or that the colonies may have been so weak that the attractant nature of the hive products was effected. The fact that there is no significant difference does not support the “trapping sink” theory, since it was expected that there would be a lower number of beetles in the apiaries with one sink colony to indicate that more beetles would be trapped in one trapped colony out of three. However, the number of beetles remaining in the two sink colony apiaries was the lowest count and contradicts this expectation (Figure 3.3).

The colony measurements of adult bees, bee brood, and honey production showed no significant differences (all $P>0.05$) over the course of the trapping period by date or when comparing apiary treatments. However, there was a significant difference ($P=0.0487$) in the number of varroa mites captured between the three apiary types, with the apiaries with two sink colonies having the lowest number of varroa mites and the no-trap control colonies having the highest number of varroa mites per day (Figure 3.4). The reason for the varroa mite numbers to be significantly higher in the control colonies is unknown at this time. Further studies may be able to discern if there is a definite correlation between the traps and lower varroa populations. There were no colonies that died during the 2010 year, but four colonies died during the 2011 experimental year. The deaths consisted of two non-trapped colonies in an apiary with one trap, a non-trapped colony from a two sink colony apiary, and a trapped colony in a two sink colony apiary. However, all but one of these colonies died early in the investigation and each one had low beetle numbers in the samplings in which each colony contained living honey bees. Therefore, these numbers would not skew the beetle counts for those apiaries. One of the non-trapped colonies in the one sink apiaries died before the final shakeout, but previous beetle counts for the colony indicate that the predicted numbers of SHB remaining would be insufficient to change the significance of the results.

The majority of the data gathered over the course of this 2 year investigation does not support the “trapping sink” theory that has been hypothesized. The lack of significance in the majority of the 24-hour surveys conducted every 6 weeks or the end of project shakeout numbers of SHB indicates that the numbers of beetles captured in the treatment apiaries compared to the control apiaries are not different. However, this contradicts previous studies in which sink colonies removed more beetles from colonies than were found in control colonies at the end of a season (Hood & Tate 2010). The lower number of SHB in the control apiaries possibly due to weather or variation in colony strength between colonies may have contributed to this lack of significance, as well as the variation present throughout the replications. Nevertheless, the biweekly data seem to support the “trapping sink” theory, which includes the biweekly SHB samplings (Figure 3.1). The lack of significant difference (all $P>0.05$) overall in the 2-week surveys indicates that the one colony in three treatment captures as many beetles as having two trapped colonies within an apiary consisting of three colonies.

As a whole, the data does not conclusively determine the validity of the “trapping sink” theory. The possibility of additional replicates during years with better conditions for beetle reproduction may provide more conclusive data in terms of significance from the controls. Traps placed in colonies within apiaries with more than three colonies may also give additional information on any effect of a possible “trapping sink” in controlling the adult SHB population. Placing traps within a colony will reduce the adult SHB population, but currently this must be done in every colony to provide a substantial effect to the population of beetles present in an entire apiary.

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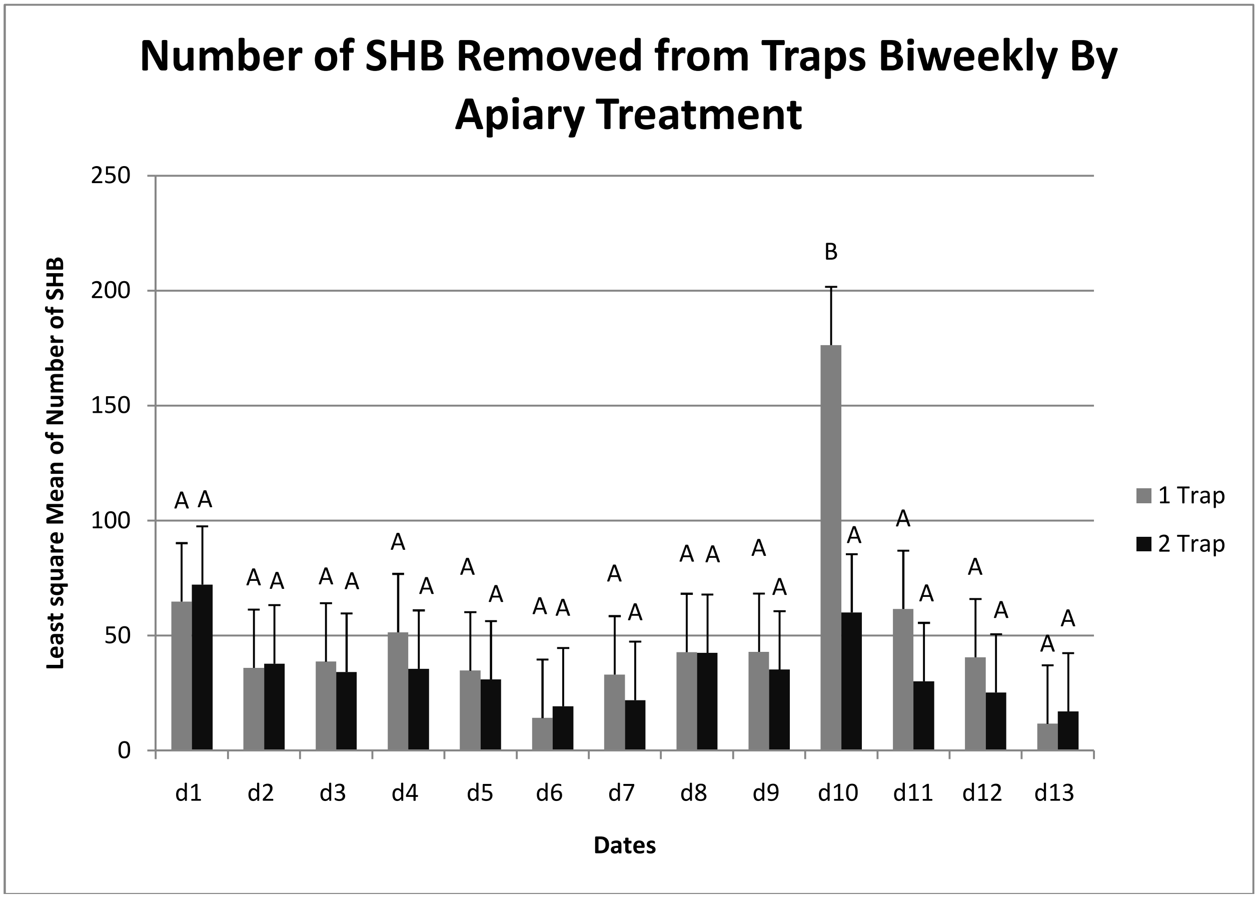

Figure 3.1: Least square mean analysis of the total number of adult small hive beetles captured in the Freeman and Cutts traps of the one colony trap and two colony trap apiaries biweekly ± standard error from variation during the experimental period in Pickens County, South Carolina for both investigative years. Horizontal axis labels indicate compiled numbers from comparable dates in both years. Different letters indicate significant differences (P<0.05) and the same letters indicate no significant differences (P>0.05).

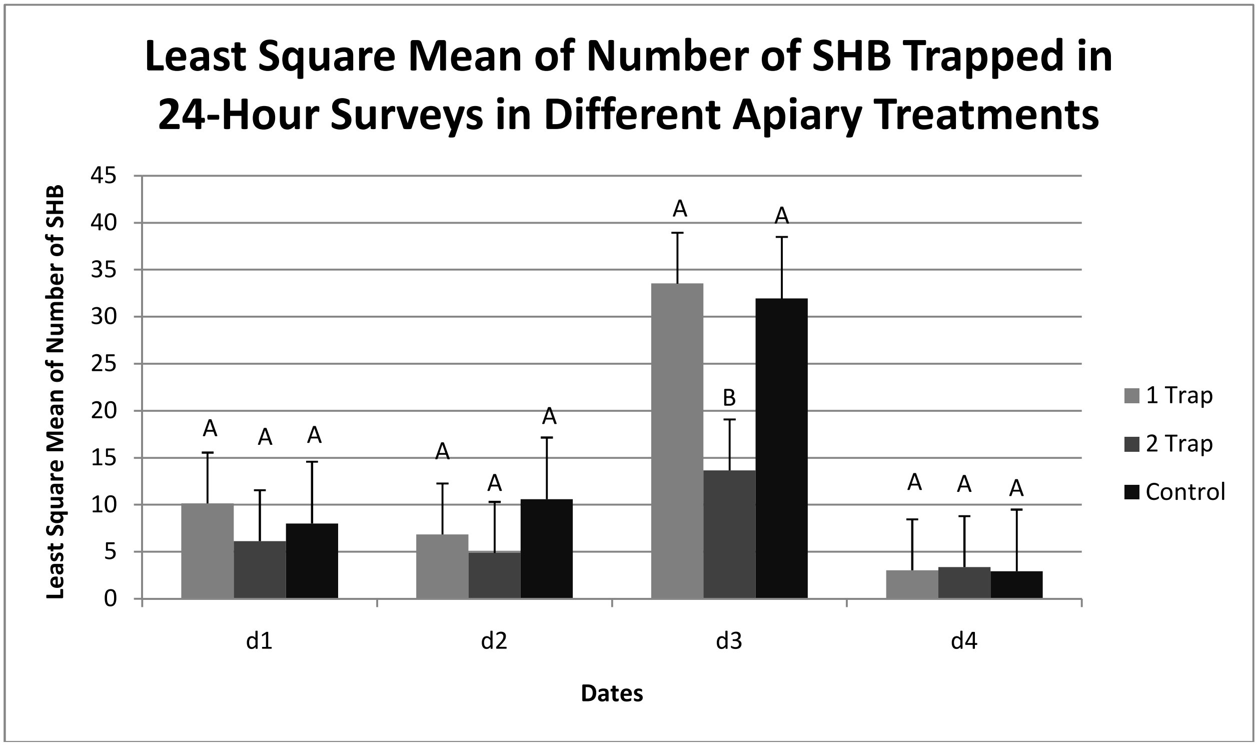

Figure 3.2: Least square mean analysis of the number of adult small hive beetles captured in the Freeman trap + standard error from variation over the 24-hour survey period conducted every 6 weeks during the trapping period for all apiary treatment types in Pickens County, South Carolina for both investigative years. Horizontal axis labels indicate compiled numbers from comparable dates in both years. The same letters indicate no significant differences (P>0.05).

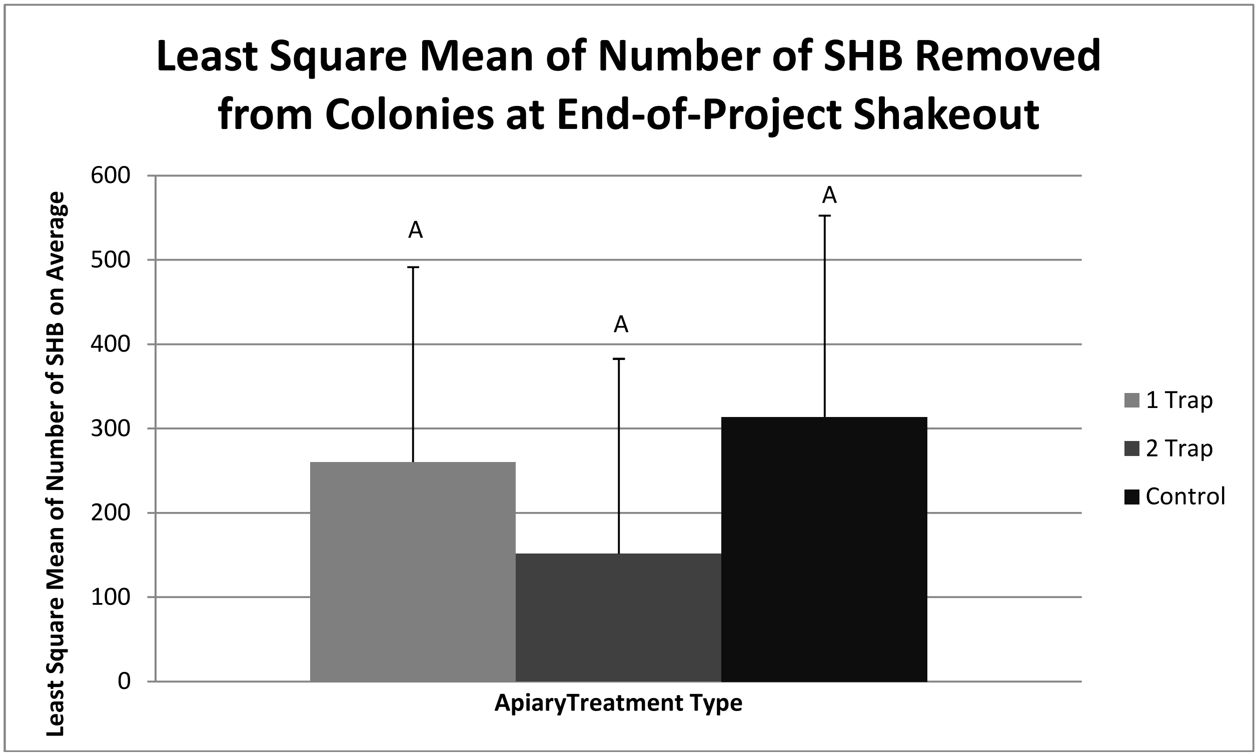

Figure 3.3: Least square mean analysis of the total number of adult small hive beetles shaken out and killed with a hive tool from colonies ± standard error from variation comparing each apiary type following the trapping period in Pickens County, South Carolina for both investigative years. The same letters indicate no significant differences ($P>0.05$).

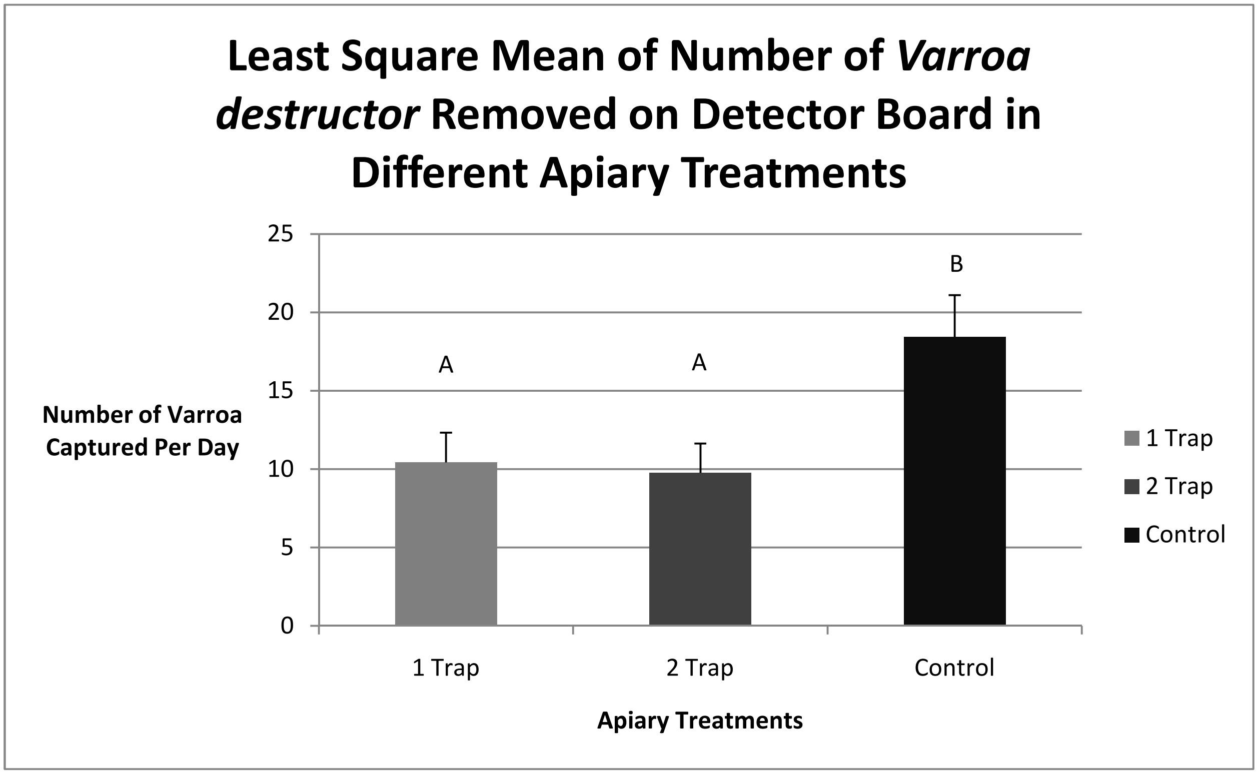

Figure 3.4: Least square mean analysis of the 3-day average number of varroa mites dropped onto a detector board every 6 weeks ± standard error from variation comparing each apiary type following the experimental period in Pickens County, South Carolina for both investigative years. Different letters indicate significant differences ($P<0.05$) and the same letters indicate no significant differences ($P>0.05$).

CHAPTER FOUR

Summary

Upon consideration of the results illustrated by the trap comparisons it can be concluded that the Freeman trap is the most effective of the three traps investigated at removing small hive beetles from a colony of honey bees. Based on the research conducted, the current recommendation would be to utilize the Freeman trap to control small hive beetles within a colony of honey bees. The Hood trap or Better Beetle Blaster may also be utilized in late summer or early fall to capture small hive beetles before they go into cluster along with the bees.

The second investigation into “trapping sinks” did not show results that suggest a definitive recommendation of the placement of colonies treated with traps within an apiary to control the small hive beetles. The lack of a discernible “trapping sink” effect in an apiary leads to the recommendation that any trapping or control method should be utilized in every colony in an apiary to maximize the effectiveness of control. Further evidence for these findings can be found in Appendices A and B, since there is a much lower number of beetles present in the trapped colonies within an apiary. This suggests that even within an apiary, the beetles are not attracted to the trapped colonies in a similar manner to the lower beetle numbers within the apiary.

In the “trapping sink” investigation, the lack of significance between the trapped apiaries and the control apiaries was at least partially due to lower beetle numbers than were expected in the control apiaries. One theory for this is that the precipitation in the experimental area was insufficient to allow for the beetles to survive as pupae within the soil, since moist soil is required to prevent desiccation during this life stage. Precipitation data to support this theory were received from the National Climatic Data Center (NCDC) and Appendix C demonstrates this information. The precipitation for the experimental area is noticeably less in the months of July and August during the two investigative years when compared to the same months of the previous years. The effect of precipitation on the small hive beetle population during these months is important because of the accumulation of adult beetles due to generational overlap. Therefore, it is possible that the lower precipitation had a partial effect on the number of beetles that emerge as adults in order to infest honey bee colonies.

Upon consideration of the methodology utilized and the results discovered in my research, there are several aspects that present the possibility for further investigation. The comparison of other commercially available traps and control options is one avenue for investigation. The Freeman trap was the most effective trap out of the three compared traps, but there may be more effective traps that have not yet been compared. A study comparing other traps would allow the beekeeper to make the most informed and economically feasible option for controlling the population of small hive beetles in honey bee colonies. Looking further into the “trapping sink” theory with various apiary configurations, such as apiaries with more than three colonies, may also clarify the results demonstrated in this research. There is also the possibility of investigating the effect of the Freeman trap on *Varroa destructor* and other honey bee pests, since the “trapping sink” investigation also demonstrated a significant difference in the number of varroa mites present in control apiaries when compared to apiaries with sink traps. Further study into the cause behind this phenomenon may lead to control options for the varroa mite as well as the small hive beetle, which could be economically important for the beekeeping community.

APPENDICES

Appendix A

Title

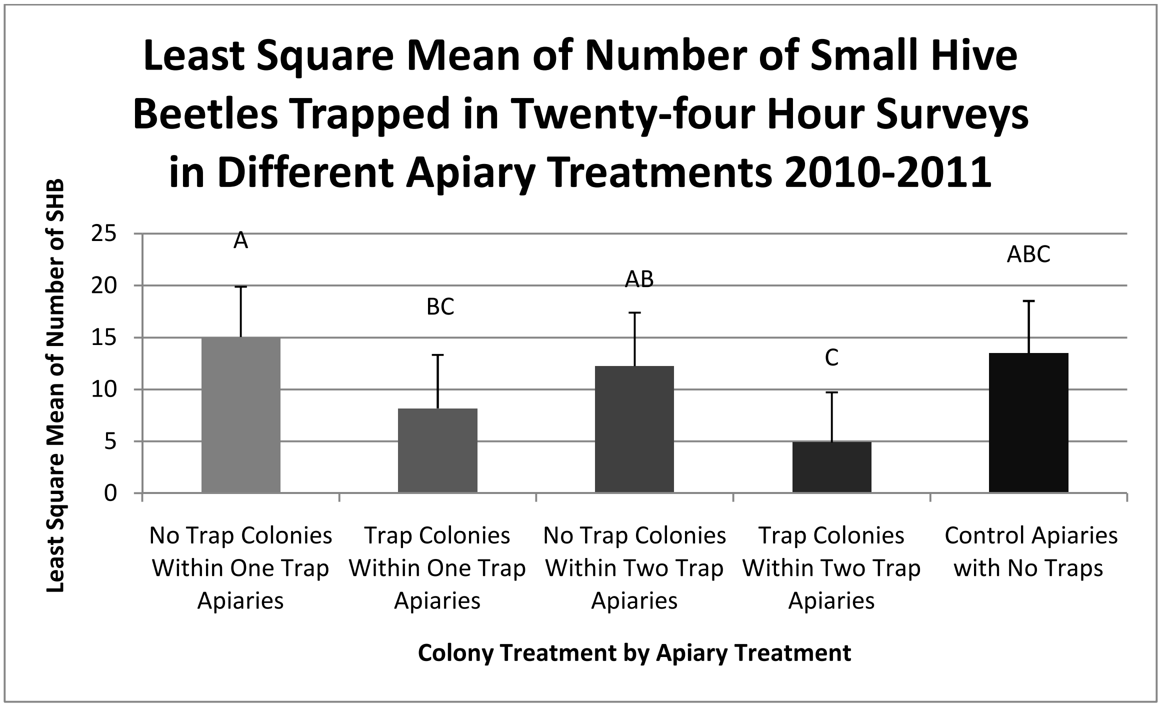

Figure A-1: Least square mean analysis of the number of adult small hive beetles captured in the Freeman trap ± standard error from variation over the 24-hour survey period conducted every 6 weeks during the trapping period for all apiary treatment types in Pickens County, South Carolina for both investigative years.

Appendix B

Title

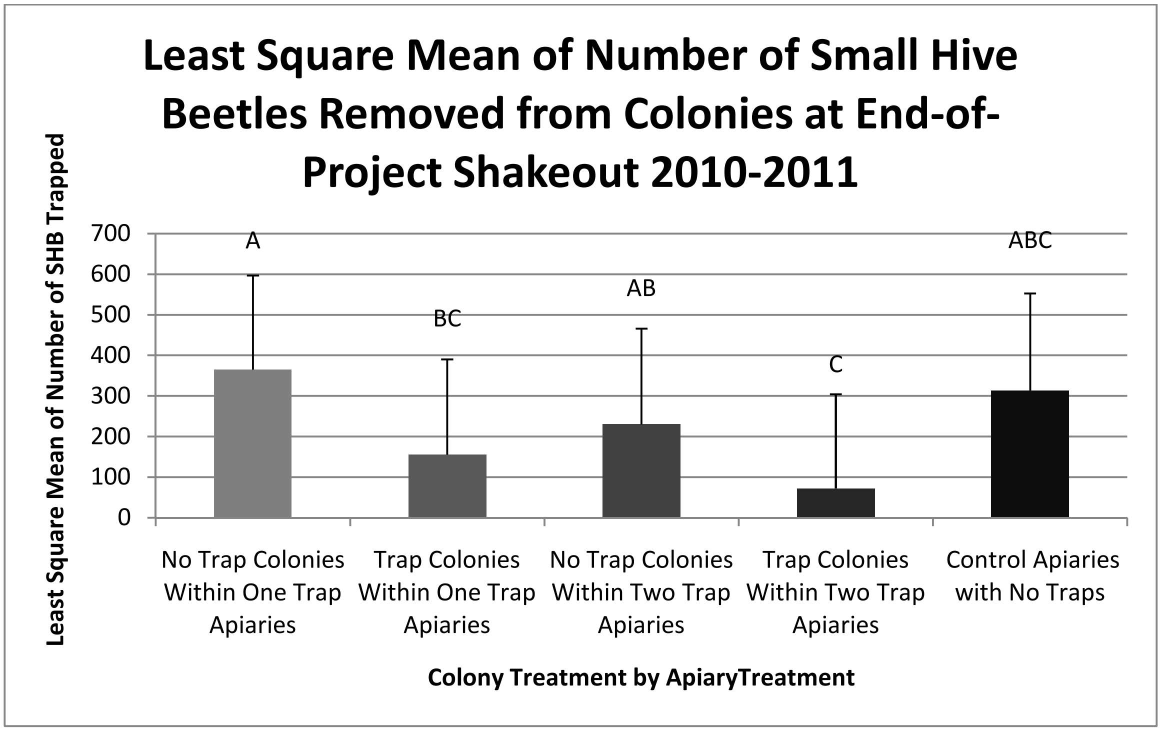

Figure B-1: Least square mean analysis of the total number of adult small hive beetles shaken out and killed with a hive tool from colonies ± standard error from variation comparing each apiary type following the trapping period in Pickens County, South Carolina for both investigative years.

Appendix C

Title

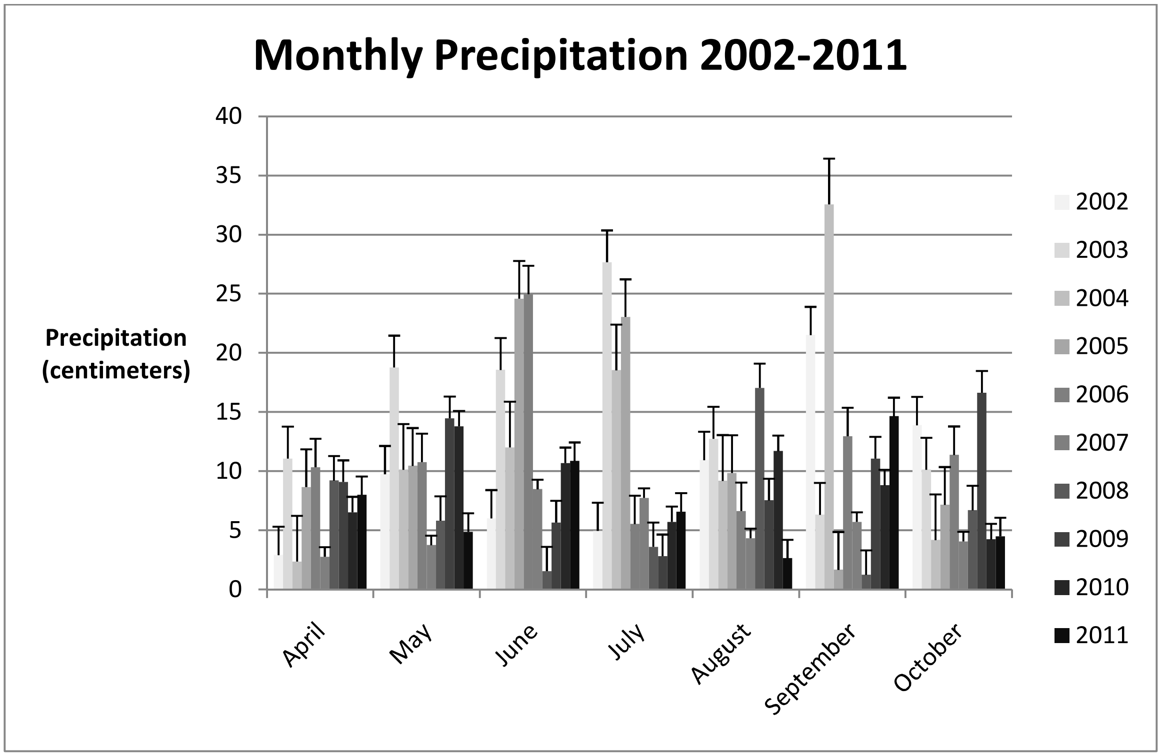

Figure C-1: Monthly precipitation at the Lamaster's Dairy Farm station in Pickens County, Clemson, South Carolina ± standard error from variation during the years 2002 through 2011.