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TRAPPING SMALL HIVE BEETLES, AETHINA TUMIDA MURRAY, INSIDE HONEY BEE COLONIES

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TRAPPING SMALL HIVE BEETLES, *AETHINA TUMIDA* MURRAY, INSIDE HONEY BEE COLONIES

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ABSTRACT

The small hive beetle, *Aethina tumida* Murray, (Coleoptera: Nitidulidae) is an economically important honey bee pest, particularly in the southeastern United States. One method of controlling this pest is the use of an in-hive trap. Many different attractants have been developed and are used in such traps. Part one of my thesis involved testing a new attractant, a pollen substitute inoculated with the yeast *Kodamaea ohmeri* (NRRL Y-30722). Thirty-two test colonies were established with 0.9-kg (2-lb) packages of bees with queens. Eight colonies were placed in each apiary, and colonies received one of three treatments: 1) yeast-based attractant 2) apple cider vinegar, a known small hive beetle attractant, and 3) control, an empty trap. The delivery system used for the test was the "Hood small hive beetle trap." Data was collected over a six-month period from May to November 2006. Both the yeast-based and the cider vinegar attractants increased small hive beetle trapping as compared to control traps. Additionally, the yeast-based attractant showed an increase in small hive beetle trapping efficiency during the warmer months of July and August. More capped brood and fewer beetles were present in colonies with attractant-loaded traps as compared to control traps.

The second year of my research involved determining the most effective location for trapping the small hive beetle within a honey bee colony. Five apiaries were established between 31 March and 2 April 2007 at approximately 2.4 km apart; each contained five honey bee colonies. Two Hood beetle traps were placed within each colony; one trap was placed in the top honey super, the other in the brood chamber. Data were collected for a seven-month period, from May to November 2007. There was no

significant difference in number of beetles captured between the two trap locations for any of the ten sampling dates. Currently, most traps developed for small hive beetle focus on trapping near the hive floor. However, my data suggest that trapping can be just as effective in honey supers above the brood chamber. Additionally, seasonal differences in trapping effectiveness were observed.

DEDICATION

This manuscript was made possible by the love and support of my wife, family and friends. For all they have done to see me through this challenge, I dedicate this work, the culmination of the past two years of my academic and research endeavors.

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CHAPTER ONE

LITERATURE REVIEW

Introduction

Overview

The small hive beetle (SHB), although a relatively recent addition to the list of honey bee pests, boasts a rapidly-enlarging habitat (Neumann and Elzen 2004). With populations on the African, North American, and Australian continents, the effect of the SHB is truly felt world-wide. The first specimens discovered in the Western hemisphere were collected in Charleston, South Carolina in November 1996; however, they were not immediately classified as SHB. Rather, samples collected in 1998 from honey bee colonies near St. Lucie, Florida were identified first (Hood 2004). Both St. Lucie and Charleston are coastal areas and possess large ports, leading some to speculate that the SHB traveled to the United States via cargo ships (Gillespie 2003). The SHB impacts colonies in a number of ways, from destroying honey crops to causing bees to abscond; the latter has been observed even in strong colonies (Hood 2004). The harm inflicted by the SHB has spurred much research concerning this insect; a variety of control methods have been developed and tested, utilizing an assortment of attractants. Further research concerning both the relative attractiveness of SHB attractants and the comparative success of traps and trapping techniques will yield significant contributions to the development of an effective control solution.

Economic Impact

In its native range the SHB is considered to be of little economic importance and usually only effects weakened colonies (Lundie 1940). While reasons behind this tolerance are still largely unknown, it is speculated that the subspecies of honey bees occurring in Africa, African bees *Apis mellifera scutellata*, and the Cape honey bee *Apis mellifera capensis*, of South Africa may be more resistant to SHB (Smith 1953, Ellis 2003). Colony management practices are also different between the Untied States and Africa, and may also contribute to tolerance (Wilson 1999). However, when SHB were accidentally introduced in the United States in 1996 major problems occurred.

In the years between their discovery and 2003, the SHB expanded to thirty different states, as well as Canada and Australia (Hood 2004, Dixon 2002, Gillespie 2003). This rapid, extensive movement is likely the result of beekeepers moving colonies into and out of infested areas (Hood 2004). Infected equipment and package bees may have played a supporting role. As in the United States, the first documentation of the SHB in Australia occurred in a coastal area, further strengthening the hypothesis that the beetles may have been transported via ship (Gillespie 2003). SHB have also been reported in Egypt. They were first detected in Etaie Al-Baroud in the summer of 2000 (Mostafa and Williams 2002). It is unclear if the SHB is native or introduced to Egypt at this time and further research needs to be done.

The impact of the SHB on honey bee colonies can range from insignificant to severe. Healthy European honey bee colonies in the United States have been shown to be killed by elevated SHB numbers (Elzen *et al*. 1999). Once a colony is heavily infested,

bees will sometimes simply leave the colony (Elzen *et al*. 1999). In its native range where the African subspecies of honey bee occur, the SHB is listed as both a scavenger and a symbiont and only occasionally damages honey bee colonies (Ellis and Hepburn 2006). Some severe infestations occur and may cause honey bees to abscond. If beetle populations are low and the bees abscond due to other factors, low beetle populations can increase rapidly and reach large numbers. These numerous larvae and adults are then classified as scavengers, cleaning up the remains of old hive products.

The European subspecies of honey bee used to pollinate our food crops and produce honey, showed greater susceptibility to the SHB. Even strong colonies of European honey bees were reported to be destroyed (Elzen *et al.* 1999). In 1998, the state of Florida alone, estimated losses from SHB to be in excess of $3 million (Neumann and Elzen 2004). These losses are mostly the result of colony destruction and damage to stored honey supers. Methods for good sanitation are available and include, extracting honey within 2-3 days from harvesting to prevent beetle damage and keeping equipment free of excess pollen and wax (Ellis *et al.* 2002c). During the first few years of beetle discovery some commercial beekeepers attributed the loss of thousands of honey bee colonies and equipment to SHB damage (Somerville 2003). Losses were also reported due to the bees negative effects on domestic and oversees packages and queen markets. Due to worry of the SHB being brought into the UK, all movement of bees from the United States into the UK was prohibited (Brown *et al.* 2002). There has also been some concern from the fruit industry regarding the SHB. It has been shown in the lab that SHB will eat and reproduce on a number of various fruits including: mango, banana, and

grapes (Buchholz *et al.* 2008), as well as avocado, cantaloupe, pineapple, orange, strawberries, papaya, and grapefruit (Eischen 1999). So far, it appears that honey bees and their products are preferred food of SHB and only go to fruit if honey bee colonies are not available. The risk to fruit crops, therefore is minimal (Eischen 1999). Small hive beetles have also been reported to successfully live and reproduce in commercial bumble bee colonies (Stanghellini *et al.* 2000, Spiewok and Neumann 2006a). It is not known if SHB seek out native bumble bees, but given the ability of SHB to locate honey bee colonies, it is possible that native bumble bees are at risk (Stanghellini *et al.* 2000). In an effort to protect our native pollinators, further investigation of SHB effects on bumble bees should be performed.

Biology of Small Hive Beetle

The small hive beetle, *Aethina tumida* Murray (Coleoptera: Nitidulidae), is a member of the Nitidulidae family. Nitidulidae encompasses a variety of sap beetles; it is distinguished from similar beetles by grooved metacoxae, dilated tarsal segments, transverse procoxal cavities, three-segmented antennal club, and small fourth tarsi (Neumann and Elzen 2004). For the purpose of self-protection, the adult beetles possess a thickened exoskeleton and the ability to pull the legs and head under the body (Ellis and Hepburn 2006). This thickened integument also makes it difficult for worker bees to grasp adults and expel them from the hive. Adult SHB reach lengths of approximately 4-5mm. Adults are dark brown to black and generally live inside honey bee colonies. Adult females slightly outnumber males and are slightly larger (Ellis 2002a).

The SHB is primarily known as a scavenger and parasite of honey bee colonies. While in the colony SHB feed on bee brood, honey and pollen (Hood 2004). It has also been shown that SHB will initiate a regurgitation response from worker bees (Ellis *et al.* 2002d). The beetles accomplish this task through their ability to mimic the stimuli bees use to receive food from one another. This has been particularly observed in beetles imprisoned by honey bees within the colony.

Small hive beetles have also been shown to utilize alternative food sources when honey bee colonies are unavailable. Studies have shown that SHB will oviposit on fruit and decaying meat. In the lab, SHB have reproduced on mango, banana, and grapes (Buchholz *et al.* 2008), as well as avocado, cantaloupe, pineapple, orange, papaya, and grapefruit (Eischen 1999). While reproductive success is much lower when alternative food sources are employed, those food sources become necessary in the absence of bee colonies. This flexibility in diet likely aids in SHB survival and allows beetles to disperse between areas where honey bees are not available (Buchholz *et al*. 2008).

Adult SHB are strong fliers and are capable of flying several kilometers (Somerville 2003). The eggs are laid in concealed areas of the hive and the larvae seek out pollen and honey for food (Pettis 2000). Eggs are generally laid in the brood area, if allowed by adult bees, and hatch after three to six days (Hood 2004). The larvae are creamy-white in color and begin to feed on pollen, brood, and honey from the bee colony. On average, larvae will continue to feed for 13.3 days until they are ready to pupate (Hood 2004). To complete the process, mature larvae crawl out of the hive entrance and enter the soil. Mature larvae will generally exit the hive in late evening from 1900 h to

2200 h; peak activity occurs at 2100 h (Hood 2004). In the sandy soil of central Florida, around 80% of the pupae were found within 10cm of soil surface; 83% were found within 30cm of hive entrance. Development time from egg to adult takes between 38-81 days, depending on weather and diet (Pettis 2000).

Control Methods

Cultural

There are many things that beekeepers can do to minimize SHB damage to their colonies as well as in the honey house. One important factor in limiting SHB damage is maintaining strong colonies. Proper treatment of mites and various brood diseases will help keep honey bee populations high and limit beetle activity (Hood 2004). Other good management practices include: limiting wax moth activity, abstaining from over-supering, check queen status, and avoid starvation. Removal of feeders when not in use may help, as SHB can seek refuge in them. Sugar patties used for various treatments should be used carefully as studies show an increase in beetle population when sugar patties are present (Westervelt *et al.* 2001). Proper apiary location can make a difference; areas that are open and sunny are preferred. Beetles reproduce better in moist soil, so it is recommended to place colonies in areas that are well drained and away from irrigation systems (Hood 2004).

In addition to colony management practices it is also important to use good sanitary practices in the honey house. When removing honey supers for extraction it is recommended to extract the honey within 2-3 days to keep SHB from damaging the

honey crop. Keeping the relative humidity below 50% will desiccate SHB eggs. Any sources of wax and pollen should be properly stored to keep SHB from reproducing in them. Pollen traps should not be left on colonies for extended periods of time as beetle larvae will feed and reproduce on trapped pollen (Hood 2004). Bleach was found to be the most effective and fast-acting household product at killing SHB larvae when tested against oil, oil and water, vinegar, and detergent. Bleach was suggested for use in the honey house or to clean infested comb (Park *et al.* 2002).

Behavioral

The race or subspecies of honey bee can affect SHB damage on honey bee colonies. It has been shown that *Apis mellifera capensis*, the Cape honey bee, and *Apis mellifera scutellata*, the African honey bee, actively remove SHB eggs and larvae (Spiewok and Neumann 2006). No significant difference in effectiveness of SHB larval and egg removal between the two races was found. European honey bees also show no significant difference in their ability to remove SHB eggs and larvae when compared to the Cape honey bee (Ellis *et al*. 2004a). The SHB will oviposit in sealed brood cells, exposed comb and cracks around the colony. Worker bees actively remove any eggs that can be reached. However, some eggs oviposited into cracks are missed. The larvae emerging from these cracks are also removed. The removal of both eggs and larvae allow for beetle control. Colonies that are stressed from disease, parasites, or high numbers of adult SHB are more likely to be overrun by beetle larvae. This is primarily caused by a reduction in worker bee number or a relocation of work bee attention away from SHB egg and larval removal (Ellis *et al*. 2004a).

In addition to removal of SHB from the colony, honey bees will also create propolis prisons and force beetles into them (Neumann *et al.* 2001). These prisons are commonly found on top bars and periphery of the colonies. It normally takes worker bees between 1-4 days to construct the prisons and bees have been observed guarding them continually for up to 57 days. While in these prisons, SHB have been observed to mate and cannibalize each other. It is also believed that the encapsulation response is not triggered until SHB levels reach a threshold level (Neumann *et al*. 2001).

Chemical

One SHB control technique is the use of a soil drench pesticide to kill beetles as they enter the soil to pupate. Gard Star® (a.i. 40% permethin, Y-Tex Corp, Cody, Wyoming, 82414, USA) has been registered in several SHB infested states since 1999 (Hood 2004). The product is mixed with water and applied to the soil in front of the colony, killing SHB pupae as they enter in soil. Check Mite + (a.i. 10% coumaphos plastic strip, Bayer Corp., Shawnee Mission, Kansas) has been available in the United States since 1999 (Hood 2004). These chemical products are registered under an emergency section 18 label by some SHB-infested states. While some chemical treatments are effective in controlling SHB, alternative non-chemical methods are preferred. In addition to accidentally killing honey bees, there is a risk of honey and wax contamination.

A trap was developed using the chemical coumaphos and tested by Elzen *et al.* (1999). Coumaphos strips were placed under cardboard strips and stapled to the hive bottom board. The beetles, constantly seeking refuge from the honey bees, go under the

cardboard receiving a lethal dose of pesticide. It was reported that in a 24-hour period two-thirds of the adult SHB on the bottom board were found dead with an additional one-quarter in the next 24 hours. The coumaphos also killed 94.2% of larvae found on the bottom board within a two-day time period (Elzen *et al.* 1999). While affective, coumaphos must be handled with care. Furthermore, coumaphos can only be applied when no honey supers intended for human consumption are on honey bee colonies. The coumaphos trap is only effective in warm months when beetles are active and away from the cluster (Hood 2000).

A refuge trap utilizing fipronil-treated corrugated cardboard has been developed in eastern Australia (Levot 2008). The device is comprised of two pieces of plastic that snap together, incasing the treated cardboard. The plastic refuge trap has a slit on one side that allows SHB to enter, while preventing bees from coming into contact with fipronil. The trap is placed on the bottom board of a colony; beetles enter to seek refuge from bee harassment. The trap was tested in 26 SHB-infested colonies and caused an estimated 62% overall beetle mortality within 6 weeks of installation and reduced the mean live adult beetle population by 96%. While fipronil is not yet approved for use in honey bee colonies, research found less than 1 $\mu g\ kg^{-1}$ of fipronil in honey taken from a colony equipped with a refuge trap over a one-month trial (Levot 2008).

Other Traps

Elzen *et al.* (1999) developed and tested a trap using baited bucket traps placed in an apiary. Traps consisted of 8-mesh hardware cloth glued across 7cm diameter holes in buckets. The buckets were placed randomly in infested apiaries and baited with a variety

of materials. These included: 1) 10 g of commercially obtained honey mixed with 5 g of commercially obtained pollen; 2) honey bees only; 3) excised piece of comb; 4) comb plus honey plus pollen; 5) honey plus pollen plus 50mL of bees; 6) un-baited control. The most attractive combination was honey plus pollen plus live adult honey bees.

Several in-hive traps have shown various levels of success. One of these traps is the Hood beetle trap (Brushy Mountain Bee Farm, Moravian Falls, North Carolina). Dr. Michael Hood, Clemson University, developed this three-chambered plastic box trap (Hood 2006, Nolan and Hood 2008). It is fastened on the bottom bar of either a brood or upper honey super frame and placed in a bee colony. The trap is filled with an attractant in the middle compartment and mineral oil on the sides to coat the beetles' feet, preventing their escape. This trap is not designed to eliminate SHB from a honey bee colony, but to keep the numbers below a threshold. Cider vinegar is a common attractant used in this trap (Hood 2006). This trap was compared to a jar type trap that attached to the underside of a hive bottom board. The jar used was a 1.15 kg or 2.5 lb honey jar with the exterior spray painted black to simulate the dark conditions inside a beehive which the beetles prefer. The jar lid was mounted to the bottom of a brood chamber with three screws and a 3.8 cm hole was cut through the lid and the bottom board of the brood chamber. The hole was positioned 14 cm from the rear wall of the brood chamber and a screen funnel was stapled into the hole with the cone end facing down into the jar. A piece of corrugated plastic was then placed over the hole to prevent bees from entering the jar, and to create a dark place for beetle harborage. The jar was filled with cider vinegar, and when compared to beetle numbers captured in the Hood beetle trap, there

was no significant difference (Hood 2006). These two traps lowered beetle numbers significantly compared to control colonies.

Another control method involved replacing the regular entrance of a hive with a ¾ inch PVC pipe located 3-4 inches above the bottom board. This method has been shown to lower beetle numbers by possibly excluding beetles from entering the colony or by trapping the beetle larvae inside the hive. However, lower bee brood numbers and accumulation of debris on the bottom board with this method made it impractical (Ellis 2002b).

A similar long-term study (8 month period), was conducted using 3.5 cm-i.d. PVC pipe positioned 20 cm above the hive bottom (Hood and Miller 2005). This study found no significant reduction in SHB numbers and reported a reduction of bee brood numbers and honey production for colonies with the upper hive entrance. It was concluded that in areas of high beetle activity that upper hive entrances were ineffective (Hood and Miller 2005).

A trap using a modified bottom board and baited with pollen inoculated with yeast *K. ohmeri* has shown success in controlling SHB. This in-hive trap consists of cutting an 18 x 14 cm rectangular opening on a standard Langstroth hive bottom, covering the opening with 4-mesh aluminum screen. Under the bottom board, a frame is constructed out of two-by-fours on three sides, with no two-by-four on the rear side of the hive bottom. Attached to this frame are runners where the trap slides in. Next, an 18 x 14 cm rectangular hole is cut into a plywood panel and matched with the hive bottom hole. This hole in the plywood is covered by attaching a Rubbermaid egg container lid

(Rubbermaid, Huntersville, NC) to the underneath side of the plywood. Two holes are cut in the Rubbermaid lid and two PCR96 well plates, tips cut off, are fitted to the lid. The egg tray holds the attractant and trapped beetles. Because it has been shown that beetles prefer dark, the egg tray was painted black. The tray is then snapped into the Rubbermaid lid (Torto 2007a).

A modified version of this trap (Torto 2007a), was also tested and placed in the top of a honey bee colony. When compared to the hive body trap, this trap caught significantly less beetles. Both traps were baited with pollen inoculated with the yeast *Kodamaea ohmeri*. Results from this study claimed to nearly eliminate beetles from colonies using the hive bottom trap. The unbaited traps did catch significantly fewer beetles than the traps baited with yeast-inoculated pollen, showing the attractiveness of the yeast-pollen mixture.

Attractants

In an effort to increase trapping effectiveness, various substances have been tested for their attractiveness to SHB. Those include freshly collected pollen, unripe honey, slumgum, and volatiles from adult worker bees (Hood 2004). Materials have also been tested for their attractiveness and lethality to SHB within a honey bee colony using a plastic trap (Hood and Miller 2003). These materials included alcohol, beer, mineral oil, honey, cider vinegar, and ethylene glycol. It was found that cider vinegar was the best attractant, while mineral oil was the most lethal.

SHB are shown to be attracted to chemicals honey bees themselves produce. Coupled gas chromatographic-electroantennographic detection isolated eight chemicals

present in living adult worker honey bees. These include isopentyl acetate (IPA), 2-heptanone, octanal, hexyl acetate, nonanal, 2-nonanone, methyl benzoate, and decanal. When tested in a dual-choice wind tunnel bioassay, the eight-component blend elicited a dose-dependent, upwind response from beetles. The eight-chemical blend also elicited a 74% upwind response compared to 84% response from approximately 150-200 worker bees (Torto *et al*. 2005). However, when used in a trap, the blend only lured 3% of beetles, as opposed to the 48% of beetles lured with living adult worker bees.

A later study found that three of the chemicals: IPA, 2-heptanone, and methyl benzoate, account for 70-80% of the honey bee alarm pheromone (Torto *et al*. 2007). As bees release the alarm pheromone to elicit a mass stinging attack to defend the colony, they are also attracting SHB. Furthermore, these three chemicals are produced by a yeast carried by the SHB. When grown on pollen, it produces honey bee alarm pheromones. Subsequently, the pheromones produced by beetles may recruit more SHB to an already infested colony. For this reason, *Kodamaea ohmeri* was developed as a SHB attractant for trapping purposes.

Yeast was further tested for attractiveness using a wind tunnel (Torto et al. 2007b). A pollen-based diet was conditioned with 100 adult virgin SHB for 1, 3, 7, and 14 days. The conditioning process was accomplished by allowing adult beetles to come in contact and feed on the pollen-based diet. This allows the yeast to be transferred from the beetles to the diet. These four time trials (1, 3, 7 and 14 days) were compared in SHB attractiveness to pollen conditioned with the yeast *K. ohmeri*. These four time trials (referred to as conditioned yeast) were also compared in SHB attractiveness to pollen diet

inoculated directly with the yeast (referred to as inoculated yeast). The inoculated yeast was made by mixing yeast with Millipore-pure™ water and pollen dough at a ratio of 1:100:1000 by weight and allowing the mixture to ferment for 5 days. Pollen conditioned with beetles for 3 and 7 days lured significantly more SHB than unconditioned pollen. Inoculated pollen dough was shown to be equal to the 3 and 7 day conditioned pollen. The 1 and 14 day conditioned pollen was only mildly attractive. When a chemical analysis was performed on pollen dough that had been conditioned for 3 days by SHB, there was an eight-fold increase in levels of 3-methyl-1-butanol and 2-methyl-1-butanol. Unconditioned pollen only contained an 8% concentration of these chemicals. The GC-EAD profiles of both male and female adult SHB showed 10 more detectable compounds given off by conditioned pollen dough and yeast inoculated pollen dough than that of unconditioned pollen.

Biological

Entomopathogenic nematodes have shown to infect *A. tumida* in the prepupal stage (Cabanillas and Elzen 2006). The commercially available *Heterorhabditis megidis* ('HO I' strain) and *Steinernema carocapsae* ('All strain) were tested (ARBICO Environmentals, Tucson, Arizona). Further research is needed to determine the amount of control offered by nematodes.

The bacteria stain PRAA4-1^T was isolated from forest soil collected from the Catoctin Mountain region in central Maryland. This strand was shown in the lab to be toxic to the SHB among other insect species. The bacteria was mixed with field-collected pollen and fed to l SHB larvae in the lab. Larval mortality was observed from

oral toxicity (Martin *et. al*. 2007). The results were preliminary and more research is needed before this bacterium can be used as a valid control device.

Fungal pathogens have been studied as a control method for SHB as well. Ellis *et. al*. (2004b) isolated two species of fungus, *A. flavus* Link: Gray and *A. niger* van Tieghem, from beetle larvae that died during pupation. Both soil fungi have been known to attack insects; however, there are drawbacks to these fungi. *Aspergillus flavus* has been shown to produce carcinogenic compounds, and both species cause diseases in honey bees. The study was unable to conclude which of the two species of fungi produced the mortality in the beetles. A study by Richards *et. al.* (2005), followed up with these two fungi, and discovered that *A. flavus* had 38% mortality on SHB, and when mixed with diatomaceous earth (an abrasive substance used to allow entry of fungi into the beetle), had a beetle mortality of 46%. Because of the detrimental effects of *A. flavus* to honey bees, plants, livestock, and humans an in-hive application is not practical, however, a soil treatment was suggested as a possible control technique in the Ellis *et. al.* (2004b) study.

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CHAPTER TWO

COMPARISON OF TWO ATTRACTANTS TO SMALL HIVE BEETLES, *AETHINA TUMIDA*, IN HONEY BEE COLONIES

Summary

The small hive beetle *Aethina tumida* Murray (Coleoptera: Nitidulidae) continues to be a persistent honey bee pest, particularly in the southeastern United States. A small hive beetle (SHB) attractant using pollen substitute inoculated with the yeast *Kodamaea ohmeri* (NRRL Y-30722) was tested in newly established honey bee, *Apis mellifera* L., colonies for its effectiveness over a 6-month period from May-November 2006. Thirty-two test colonies were established with 0.9-kg (2-lb) packages of bees with queens. Eight colonies were placed in each apiary, and colonies received one of three treatments: 1) yeast-based attractant 2) apple cider vinegar, a known SHB attractant, and 3) control, consisting of an empty trap. The delivery system for the test was the "Hood small hive beetle trap." Both the yeast-based (56.2%), and the cider vinegar attractants (43.4%), increased SHB trapping compared to control traps (0.3%). The yeast-based attractant showed an increase in SHB trapping efficiency during the warmer months, July and August. There were more capped brood and fewer beetles in colonies with attractant-loaded traps as compared to control traps having no attractant or mineral oil. There was no significant difference in the effect of the two attractants on capped brood and colony beetle numbers.

Keywords: *Apis mellifera*, honey bee, *Aethina tumida*, small hive beetle, pest control, beetle trapping, attractant.

Introduction

The small hive beetle *Aethina tumida* Murray (Coleoptera: Nitidulidae) is a scavenger beetle of honey bees, *Apis mellifera L.* Small hive beetles (SHB), indigenous to southern Africa, were first collected in the United States by a hobbyist beekeeper in Charleston County, South Carolina, in 1996. However, they were not identified until 1998 (Hood 2004). The original collection was made in the city of Charleston, adjacent to the Charleston international seaport, which is likely one of the ports of entry for SHB in the United States. SHB were also reported in Georgia and Florida within two years of the Charleston collection, probably introduced through seaports in these states as well. After these initial reports, SHB quickly spread to twenty-five new states by 2003 (Hood 2004). The rapid expansion of SHB throughout the United States is probably the result of movement of infested hives, equipment, and package bees. In 2002, SHB was also reported in Australia (Hood 2004). The location of this infestation was also in close proximity to a major seaport.

In the beetle's native range, south of the Saharan desert in Africa, the SHB is of little economic importance and threatens only weakened or stressed colonies (Lundie 1940). However, in the United States, particularly the warmer southeastern states where the SHB can easily overwinter, the SHB poses a greater threat (Hood and Miller 2003). The SHB negatively affects honey bee colonies by eating unprotected bee brood, eggs,

honey, and pollen (Swart *et al*. 2001). Honey bee absconding has been observed in bee colonies with high beetle numbers (Hepburn and Radloff 1998). Beetle larvae destroy honey supers and, when present in honey, render the honey unfit for human consumption.

One natural defensive behavior expressed by honey bees is the imprisonment of SHB (Neumann 2001). The worker bees harass the adult beetles and confine them in the peripheral areas inside the hive. Two pesticides, Checkmite +® (10% coumaphos AI) and Gard Star® (a.i. 40% permethrin, Y-Tex Corp, Cody, Wyoming, 82414, USA), have been developed and registered in most SHB-infested states. However, non-pesticidal control methods are needed to control this pest, especially in areas where SHB is known to cause problems. Trapping methods have been developed with various levels of success. Elzen *et al*. (1999) developed a trap, using a 15x15 cm piece of corrugated cardboard with one side stripped off to expose the corrugations. A 10% coumaphos plastic strip was stapled to the corrugations and then placed on the hive floor. The cardboard creates a place for the beetles to hide where they come in contact with the insecticide (Hood and Miller 2003). Another trap marketed for SHB control is the "West Beetle Trap", sold by Dadant & Sons, Inc. (51 South 2nd Street, Hamilton IL 62341-1397, USA). It uses a plastic tray filled one-fourth with vegetable oil and placed directly on top of the bottom board of a hive. A spacer is included to raise the hive body 2 cm to give clearance for the tray. A slotted cover with holes 2 x 45 mm allows beetles, but not bees, to pass through the openings into the oil. The hive must remain level to prevent the vegetable oil from spilling, and rain must be prevented from entering the hive to prevent

oil overflow from the plastic tray. One added benefit of this trap is that varroa mites (*Varroa destructor*) can also be killed in the oil when they fall from the colony.

None of the traps developed to date have provided total SHB elimination from honey bee colonies, but efficient trapping may keep the beetles below an economic threshold. The discovery of better attractants will increase the effectiveness of already developed SHB traps. SHB, like most other members of the family Nitidulidae, are found near fermenting or souring plant fluids (Triplehorn and Johnson 2005), making both cider vinegar (Hood and Miller 2003) and yeast-inoculated pollen substitute attractant (Torto et. al. 2007) potentially effective choices.

We report here an investigation to compare the effectiveness of two SHB attractants, cider vinegar and yeast-based attractant, placed within the Hood beetle trap inside honey bee colonies over a 6-month period. We hypothesize the yeast-based attractant will be more effective than cider vinegar in attracting small hive beetles due to yeast being associated with small hive beetles and releasing attractive odors (Torto et. al. 2007). Colony strength was measured to investigate the effects of trapping and small hive beetle populations on honey bee colony strength.

Materials and Methods

Four apiaries were established on 1 April 2006 from 0.9-kg (2-lb) packages of honey bees (Wilbanks Apiaries, Claxton, Georgia, USA). The four apiaries were located at least 16 km apart in Anderson, Bamberg, Barnwell, and Pickens Counties, South Carolina. Each apiary contained eight test colonies, each housed in a 10-frame

Langstroth beehive and honey super, for a total of thirty-two colonies. Colonies were spaced approximately 0.76 meters apart with one apiary located in full sun and the other apiaries located in partial shade during the day. Along with natural infestation of SHB, 100 lab-reared adult beetles were added to each colony on 18-19 May 2006 to ensure beetles were present in each colony, as one of the test yards had low beetle numbers.

The Hood beetle trap (Brushy Mountain Bee Farm Inc., 610 Bethany Church Rd. Moravian Falls, North Carolina 28654 USA) was used to deliver the attractants and trap SHB. One trap was placed in each colony on 27 April 2006. They were placed in either the first or the tenth frame position of the brood chamber. The traps were attached to the bottom bar of a new brood chamber frame, having no foundation, with two 10.16 cm x 1.27 cm screws. The Hood beetle trap is a plastic box that has three separate compartments. The middle compartment was filled to eighty percent capacity with attractant. Colonies within each apiary were randomly selected to receive a trap with either no attractant as a control, apple cider vinegar (White House®., National Fruit Product CO., INC. Winchester, VA 22604-1240), or USDA patented yeast-based attractant (US Patent 20060141904). In total, ten colonies were control, eleven contained vinegar, and eleven contained the yeast-based attractant.

To produce the yeast-based attractant, the yeast *Kodamaea ohmeri* (NRRL-30722) was used (Center for Medical, Agricultural and Veterinary Entomology, USDA, Agricultural Research Service, 1600/1700 SW 23rd Drive, Gainesville, Florida 32608). Per the instructions, 17.25g powdered yeast was added to 172.5ml distilled water and mixed. Pollen patties (Global Pollen Patties® 4% pollen; Betterbee, Inc., 8 Meader Road,

Greenwich, New York 12834) were then prepared for inoculation. After removing the paper coverings from the patties, 1725g was measured and placed in a pan. The water/yeast mixture was then poured over the pollen patties and placed in an incubator at 26.6 °C for three days to cure. The cured attractant was placed into the appropriate traps with any remainder refrigerated at ~2.2 °C. until needed.

The two side compartments were half filled with food grade mineral oil (Mineral Oil, U.S.P., packaged by: Cumberland Swan Smyrna TN, 37167). The mineral oil appears to disrupt the beetle's ability to overcome the fine-edged slanted lip on the inside of the trap opening. The trapped beetles eventually drown in the mineral oil. The control traps contained no attractant or mineral oil.

At 3-4 week intervals, the traps were removed for SHB counting and replaced with new traps with fresh attractant and new mineral oil. Traps were transported to a laboratory for evaluation. Honey bee colony strength, colony beetle numbers, and trap beetle numbers were recorded. Colony strength was determined by measuring 25 cm^2 of capped brood. Each brood chamber frame was examined and capped brood area was estimated by placing a scribed 25 x 25 cm^2 piece of plexiglass over each side of the frame and counting squares of capped brood. Each square was counted as one unit. The total number of units of brood counted for each colony was used as an estimate of overall colony strength.

To estimate the colony SHB population (relative to the other colonies), the inner cover was removed from the hive and bounced two times on the hive to initiate SHB adult movement. The beetles found on the bottom of the inner cover were counted. Next,

five frames were removed from the brood chamber. The number of beetles counted on the bottom board and the three exposed walls of the internal side of the brood chamber were added to the number found under the inner cover.

Data were analyzed by a randomized block repeated measures design analysis of variance (ANOVA), recognizing attractant type (yeast-based attractant, cider vinegar, or control) as main effects and apiary locations as block effects. Means were separated with least significant difference test and differences were accepted at $P \leq 0.05$. All analyses were conducted using the software package SAS (SAS Institute 1992).

Results

Figure 2.1 shows the least squares mean number of small hive beetles caught in yeast-based attractant, cider vinegar, and control traps on each sampling date. A significant increase ($P<0.05$) in trapped beetles was recorded in colonies having the yeast-based attractant as compared to the cider vinegar on the August 15th sampling date. The control colonies yield significantly less trapped beetles then both attractants on all sampling dates ($P<0.05$). The number of beetles observed in each colony on each sampling date is recorded in Figure 2.2. On the 25 July, 15 August, and 7 September sampling dates, there were significantly higher ($P<0.05$) numbers of beetles in the control colonies than in the yeast-based attractant and cider vinegar colonies. Colony strength as measured by 25 cm^2 capped brood cells was not significantly different ($P \geq 0.05$) (Figure 2.3). Table 1 lists the least square means ± SE for trapped SHB numbers, colony strength, and colony beetle sample estimates.

Discussion

The yeast-based attractant containing strain *Kodamaea ohmeri* (NRRL-30722) proved to be a more effective beetle attractant during the warmer months of this trapping investigation. While there was no significant difference in SHB trapped in cider vinegar versus the yeast-based attractant during the cooler months of the study, the yeast-based attractant did prove to trap higher numbers than the cider vinegar on most months and significantly higher numbers ($P<0.05$) on 15 August sampling date. As the year progresses, SHB numbers normally continue to build through the summer and early fall months (Figure 2.2). If increased numbers of SHB are eliminated from the population during this time, beetle numbers might be kept below a critical economic threshold.

Neither cider vinegar nor the yeast-based attractant had a negative effect on brood production, as there was no difference in brood numbers between the two attractants and the control (Figure 2.3). Reasons for colony decline from trapping techniques could be from volatiles released from the two attractants. In most observations, the brood numbers were numerically higher in test colonies having either of the two attractants when compared to the control colonies. Lower beetle numbers may have alleviated colony stress, thereby contributing to the higher brood production.

The yeast-based attractant held an advantage over the cider vinegar during the hot summer months when, in some colonies, the cider vinegar evaporated before the 3-4 week time period. Evaporation could potentially be reduced through the addition of agar or other thickening agents; however, further testing is required. During this warmer time of the year, the yeast-based attractant would be the attractant of choice because of its

stability. However, the yeast-based attractant, with consistency of a pollen patty, made loading and removal of attractant difficult. Another potential drawback of the yeast-based attractant is that it must be mixed and cured before it is ready for use. Furthermore, we noted several instances in which SHB reproduced in the yeast-based attractant. Although dead SHB larvae were found in the attractant and the mineral oil, additional research is needed to determine the survivability of these larvae. No SHB reproduction was observed in vinegar. While the yeast-based attractant proved more effective than the cider vinegar on one sampling date, no significant difference in effectiveness was discovered overall. These tests were conducted with the trap placed in the brood chamber, where beetles are observed more often. Additional research is needed to investigate trapping efficiency when traps with attractant are placed in a top super or when multiple traps are used in a colony.

Currently, the yeast-based SHB attractant is not commercially available in the United States. The two attractants tested in this study appeared to reduce the SHB populations in honey bee colonies. Presently, no economic threshold for adult SHB in a honey bee colony has been published. Once a threshold is established, trapping techniques can be used to maintain the SHB population below the economic threshold or can be used as a colony SHB sampling tool. An inside-hive attractant that is too strong may attract beetles from outside the apiary, resulting in an unintentional increase in beetle numbers within colonies containing the strong beetle attractant. Further research concerning potential SHB attractants is necessary to fully capitalize on the benefits of beetle trapping. A correlation between beetle trapping and honey bee productivity and

survivability would also provide valuable insight regarding positive effects of beetle trapping in a honey bee colony.

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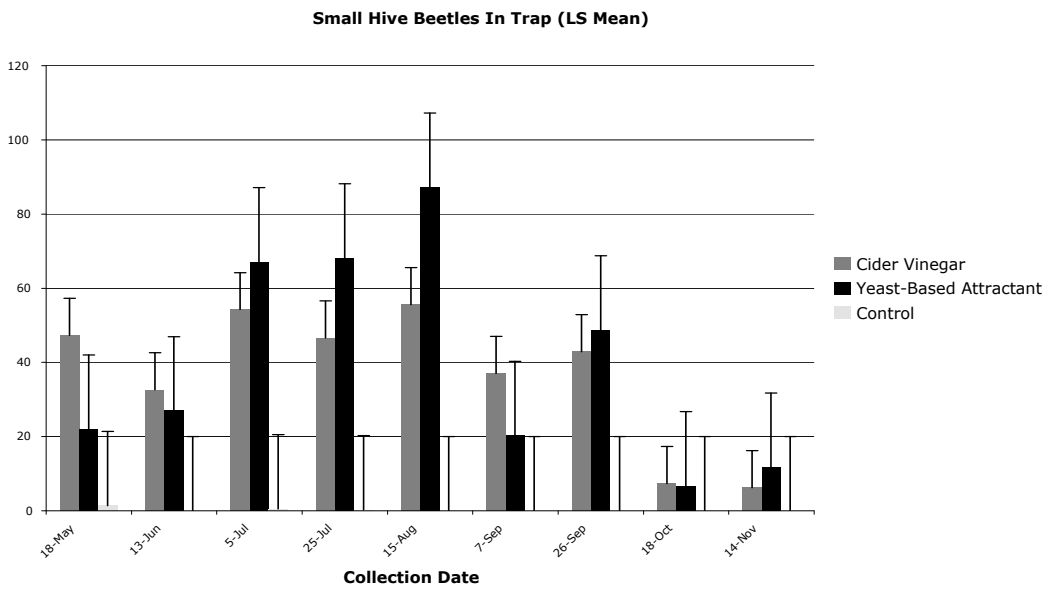

Figure 2.1: Least square mean of number of small hive beetles caught in traps. Significant difference between cider vinegar and yeast-based attractant noted on 15 August ($P<0.05$). Significant difference between control and both attractants observed on all sampling dates ($P<0.05$).

Attractant	Trapped SHB	Capped Brood (25cm²)	Colony beetle population
Yeast-Based	48.65 ± 6.15 a	152.88 ± 5.89 a	4.68 ± 1.32 a
Cider Vinegar	45.17 ± 6.46 a	151.53 ± 5.85 a	4.51 ± 1.31 a
Control	0.30 ± 6.92 b	139.94 ± 6.27 a	7.63 ± 1.41 a

Table 2.1: Least square mean trapped SHB, capped bee brood, and estimated colony beetle population size. Numbers for yeast-based attractant, cider vinegar, and control averaged over the entire study from May to November 2006. Values followed by different letters are significantly different ($P<0.05$).

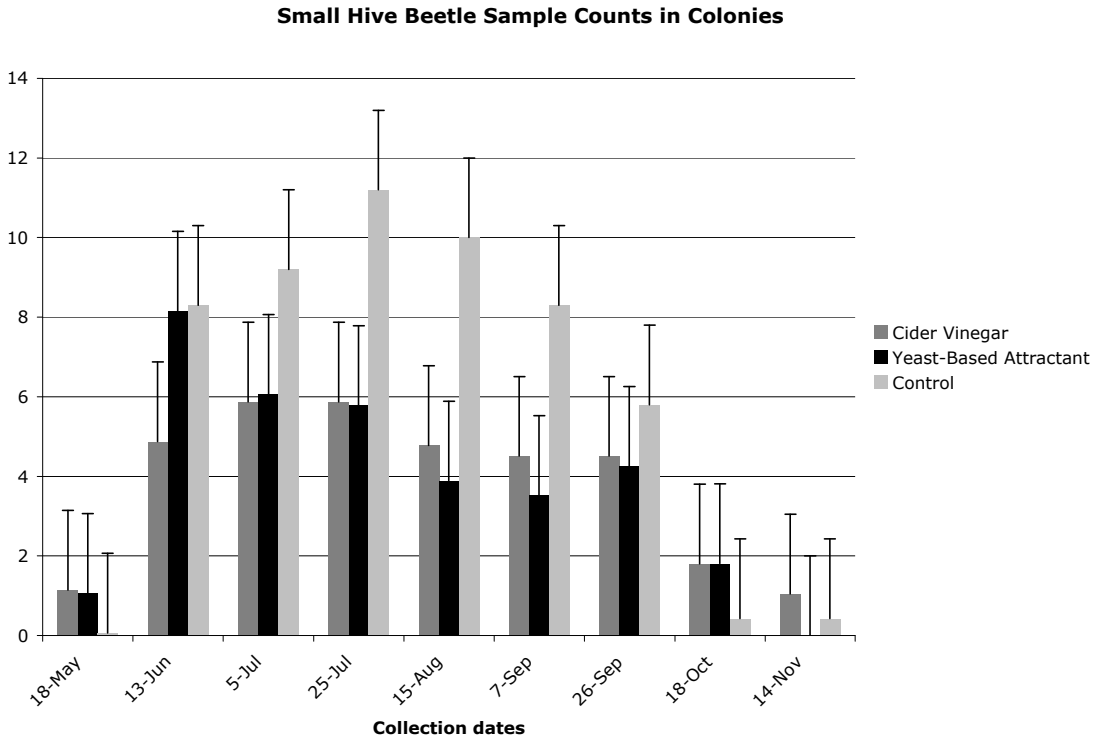

Figure 2.2: Least square mean number of small hive beetles in honey bee colonies; significant differences between control and attractants on 25 July, 15 August, and 7 September ($P < 0.05$)

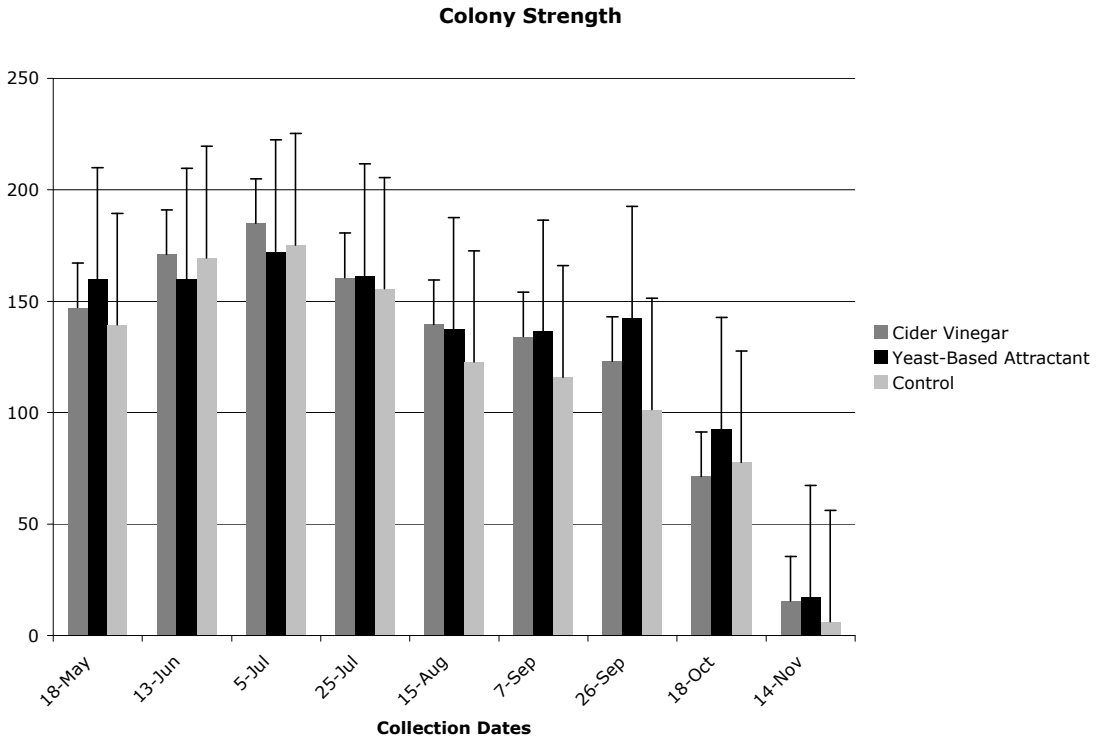

Figure 2.3: Mean number of honey bee brood observed in test honey bee colonies from May to November 2006. Capped bee brood measured by adding total number of 25 cm^2 per colony. No significant differences were found ($P<0.05$).

CHAPTER 3

TRAPPING SMALL HIVE BEETLE, *AETHINA TUMIDA* MURRAY (COLEOPTERA: NITIDULIDAE) IN HONEY SUPERS & BROOD CHAMBERS OF HONEY BEE COLONIES

Summary

To develop an effective, safe, and user-friendly trap for the small hive beetle, *Aethina tumida* (Murray), the vertical distribution of this pest within a honey bee, *Apis mellifera,* colony was examined by observation sampling and trapping in the top and bottom of honey bee colonies over a 7-month period. Two Hood beetle traps per colony were used to monitor beetle numbers, one in the top honey super and one in the brood chamber of twenty-five honey bee colonies. There was no significant difference in number of beetles captured between the two trap locations for any of the ten sampling dates. Most small hive beetle traps that have been developed focus on trapping near the hive floor. Our data suggest that trapping can be as effective in honey supers above the brood chamber, although slight seasonal differences in trapping effectiveness occurred.

Keywords: *Apis mellifera,* honey bee, *Aethina tumida* Murray, trapping, beetle sampling, pest control

Introduction

Since the small hive beetle (SHB), *Aethina tumida* (Murray), was first collected in the United States in Charleston County, South Carolina, by a hobbyist beekeeper in 1996 (Hood 2004), the SHB has spread over much of the United States, and has the potential to become a serious pest in many beekeeping operations. The SHB has been documented in the southeastern United States to affect even strong honey bee colonies (Hood 2004). To combat this new honey bee pest, various traps have been developed with no convenient device found (Hood and Miller 2005). Although several SHB traps have been developed, few have been field-tested and compared.

One control technique using a 10% Coumaphos strip (Bayer Corp, Shawnee Mission, Kansas, United States) significantly reduced SHB adults and larvae (Elzen et. al. 1999). The trap used a 15x15cm piece of corrugated cardboard with one side stripped off exposing the corrugations. It was positioned on the rear-floor of the bottom board, creating a dark environment attractive to the SHB adults and larvae. A 10% Coumaphos strip was stapled to the corrugated side of the cardboard and placed Coumaphos side down on the hive floor in the rear of the colony. The trap was stapled down to prevent bees from removing it. This method proved to be effective in killing both larvae and adult SHB, with one trial having a 94.2% mortality rate. However, the use of insecticides around and within honey bee colonies should be used as a last resort and non pesticide methods are preferred. When using insecticides, there is risk of honey and wax contamination, beekeeper exposure to the toxic material, risk to bee health, and possible development of insecticide resistance following repeated applications.

A study using the pesticide fipronil (300mg L^{-1}; 1.5mL of REGENT® 200SC L^{-1} of water), housed in a plastic harborage trap, reduced the mean adult beetle numbers in infested honey bee hives by 96% and caused an estimated 62% overall beetle mortality (Levot 2008). Fipronil is not registered for use within honey bee colonies at this time; however, this study showed that within the confines of the plastic harborage trap, the mean fipronil residues found in honey after one month of treatment did not exceed 1µg kg^{-1} and no ill effects to honey bee health were reported. The harborage device was constructed from two plastic halves that snap together holding the fipronil treated cardboard, and placed on the bottom board of a bee hive. The plastic harborage had a slit large enough for SHB to enter while excluding honey bees. SHB sought refuge within the harborage where they come in contact with a lethal dose of fipronil.

Various materials have been tested for effectiveness in attracting the SHB into traps (Hood and Miller 2003). These materials consist of alcohol, beer, ethylene glycol, mineral oil, honey, and cider vinegar. A plastic reservoir box trap was attached to a solid bottom bar of a brood frame, using two screws. The results showed cider vinegar as the most attractive and mineral oil as the most lethal. An improved three reservoir plastic trap, the Hood beetle trap, was developed and uses cider vinegar as an attractant and mineral oil as a killing agent (Nolan and Hood 2008). Vinegar and mineral oil were placed in the middle and side reservoirs, respectively. Beetles were lured into the trap, encountered the mineral oil, and could not escape through the lid opening.

The Hood trap has also been baited with a pollen/honey mixture inoculated with the yeast *Kodamaea ohmeri* (NRRL Y-30722), which is derived from the SHB (Torto *et*

al. 2007a); (Nolan and Hood 2008). While this trap does not completely eliminate SHB from a colony, it may help maintain the beetle population below an economic threshold.

A trap developed from a modified bottom board significantly reduced the number of SHB found within honey bee colonies (Torto *et al*. 2007b). This trap required a hole be cut in the hive bottom with the trap fitted to a container under the hive. The trap was baited with the yeast *Kodamaea ohmeri* (NRRL Y-30722). This study also compared beetles caught in the hive-bottom trap versus a different trap located in an empty top honey super. Eight colonies per apiary were selected for testing, four colonies with the bottom-style trap and four colonies with the top honey super trap. The bottom trap captured significantly more SHBs than did the top trap.

Hive entrance modifications have also been studied as a possible SHB control method, trapping SHB larvae within the hive and preventing their exit from the hive to pupate in the soil. Upper hive entrances consisting of 2 cm-i.d. PVC pipe, placed 7.6-10.2 cm above the bottom board in place of a regular hive entrance, have been developed and tested (Ellis et al. 2002). While SHB numbers decreased using the PVC pipe entrance, the colonies had lower brood numbers, along with a buildup of water and debris within the hive that negated the positive effects of lower SHB numbers. Attempting to alleviate the buildup of this debris caused by the PVC entrance, a separate study using screened bottom boards was conducted (Ellis et al. 2003). Results showed that even with screened bottom boards there were still more negative side effects from the PVC pipe entrance. A long-term study was also conducted using 3.5 cm-i.d. PVC pipe positioned

20 cm above the hive bottom (Hood and Miller 2005). This study confirmed a reduction of brood numbers for colonies with the upper hive entrance.

The present study used the Hood beetle trap as a SHB control device, by placement of one trap in the top-most super and another in the brood chamber. A comparison was made of captured beetle numbers from top honey super traps versus brood chamber traps. Correlation analysis was preformed between number of SHB trapped in top honey super and SHB sampled from the inner cover. Brood chamber SHB sampling numbers and number of beetles trapped in brood chamber were also investigated for correlations. Our hypothesis was that more adult beetles would occur in the bottom of a colony where food resources are more abundant and available for beetle reproduction.

Materials and Methods

Five apiaries were established between 31 March and 2 April 2007. Apiaries were located in the Clemson Experimental Forest in Pickens and Oconee Counties, South Carolina, United States. Apiaries were spaced approximately 2.4 km apart and consisted of five honey bee test colonies. All apiaries were in partial shade. Each colony was started from 0.9-kg (2-lb) packages of honey bees and a mated queen (Wilbanks Apiaries, Claxton, Georgia, United States). Colonies were housed in a 10-frame Langstroth beehive with a honey super. Colonies in each apiary were spaced approximately 0.76 meters apart in a strait line, with each colony facing the same cardinal direction. Although natural SHB immigration likely occurred from nearby apiaries, 150 lab-reared

beetles were released into the two end colonies and middle colony, for a total of 450 beetles added to each apiary. The SHB were introduced on 3 May for two apiaries and on 4 May for the other three apiaries. Beetles were introduced on the top bars of the upper-most honey super and covered with an inner cover to prevent beetle escape. The additional beetles in each apiary increased the likelihood of beetles trapped.

On 1 May, Hood beetle traps (Brushy Mountain Bee Farm Inc., Moravian Falls, North Carolina, United States), were randomly installed in all 25 colonies in either the first or tenth frame position of each brood chamber and top honey super. The Hood beetle trap was attached to the bottom bar of new frames with two pan-head sheet metal screws (#6 x 12.7mm). The Hood beetle trap was a plastic box with three separate compartments. The middle compartment was filled to 80 percent capacity with attractant, cider vinegar (White House®., National Fruit Product Co., INC. Winchester, United States). The outer two compartments were filled to 40 percent capacity with mineral oil, used as a killing agent (Mineral Oil, U.S.P., packaged by: Cumberland Swan Smyrna, Tennessee, United States).

Each colony was serviced on 15 May, 1 June, 15 June, 6 July, 27 July, 17 August, 7 September, 28 September, 29 October, and 19 November. During servicing, traps were replaced with traps containing fresh attractant and mineral oil. Colony strength was determined by counting the number of 25 cm^2 of capped brood. Each brood chamber frame was examined for capped brood, using a sheet of Plexiglas (DOW®, Midland, Michigan, United States), scribed with 25 cm^2 placed over each side of the frame. Each 25 cm^2 of brood was counted as one unit of brood. The total number of brood units was

used to estimate colony strength. Adult beetles were counted from two areas of each colony. The inner cover was removed from the hive and bounced two times on the hive to initiate SHB movement. The beetles on the bottom of the inner cover were counted. Next, five adjacent frames from one side of the brood chamber were removed. The number of adult beetles counted on the bottom board and the three exposed walls of the interior sides of the brood chamber were added together for a second count. The inner cover and brood chamber beetle counts were used for beetle population comparison within the hive.

Data were analyzed based on a model for a randomized block with repeated measures. Analysis of variance (ANOVA) was used to determine significance of trap location, beetle sampling location, and sampling dates. Means were separated with Fishers least significant difference test, with $P \leq 0.05$ considered significant. Correlations were done using Pearson correlation coefficients, with $P > 0.05$. All analyses were conducted using the software package SAS (SAS Institute 1992).

Results

There was no overall significant difference in number of SHB captured in traps in the top honey super and those in the brood chamber during the 7-month investigation (Figure 3.1). While no significant differences were observed, more beetles were caught consistently in the super traps during late summer through early fall, where as more beetles were caught in the brood chamber traps during early to mid-summer. Over the 7-month study a total of 12,705 SHB were trapped in the top honey super and 12,505

beetles were trapped in the brood chamber. Figure 3.2 compares the mean number of beetles counted under the inner cover and the mean number of beetles counted in the brood chamber. Significant differences were found between all sampling dates except 1 June, 17 August, 7 September, and 19 November (Figure 3.2). Between 15 May and 28 September, average colony strength fluctuated between a high of 160 cm^2 and a low of 96 cm^2. Following 28 September, brood numbers decreased to a low of 11 cm^2 on 19 November. No correlations were found between the number of SHB sampled from the inner cover and SHB trapped in the top honey super (Pearson's correlation $R=0.04$, $n=50$, $P>0.05$). Brood chamber SHB sampling numbers and number of beetles trapped in brood chamber also showed no correlations (Pearson's correlation $R=0.14$, $n=50$, $P>0.05$).

Discussion

Trapping data showed no significant difference between trapped SHB numbers in the top-most super and in the brood chamber. This information can be applied when developing a trapping system for this pest. Torto *et al*. 2007b found that a trap in the bottom of a honey bee colony caught significantly more beetles than a trap in the upper regions of the hive during 4-week and 7-week trials. However, having different trap designs for the top and bottom traps may explain the discrepancy between their results and our investigation. Our study used identical traps in the top honey super and brood chamber. Our research suggests that trapping SHB in the upper area of a honey bee colony is as effective as trapping in the brood chamber when using the Hood beetle trap with cider vinegar as an attractant and mineral oil as the lethal agent. In addition to being

equally effective, the convenience of servicing traps in the top-most honey super, when compared with the brood chamber, should be considered. To maximize the number of SHB removed from a colony, a beekeeper is advised to place traps in both the top and bottom of a hive.

Counting beetles under the inner cover and in the brood chamber suggested an increase in beetle numbers as the season progressed until 27 July when the maximum number of beetles was recorded (Figure 3.2). After 27 July, both the inner cover and brood chamber sampling numbers began to decrease, suggesting a decline in the SHB adult population. However, in the brood chamber sample on 28 September, there was an increase in beetles. This increase in number of beetles occurred one sampling date before the drastic increase in both super trap and brood chamber trap numbers on 29 October (Figure 3.1). While the number of beetles counted under the inner cover was consistently lower than the number of beetles counted in the brood chamber throughout the study, the inner cover did not have the large increase in beetle numbers on 28 September that occurred in the brood chamber. The trapping increase could be explained partially by the extra week the traps were allowed to remain in the colonies between 28 September and 29 October. The previous four samplings were conducted at 21-day intervals while the time between 28 September and 29 October date was 28 days. While this is one possible explanation for the increase in trapping numbers, it does not explain the increase in beetle numbers in the brood chamber on 28 September, or the reason for the inner cover beetle count to remain low. One possible explanation could be a single colony outlier. Colony number 20 had a count of 65 beetles in the brood chamber for 28 September. With this

colony removed from the average, the mean number of beetles in the brood chamber drops from a mean of 15 beetles to a mean of 13. With this decrease, beetle numbers were similar to the data collected in another study in 2006 (Nolan and Hood 2008). Inner cover and brood chamber SHB counts from our present study (Figure 3.2), and from a similar study in 2006 (Nolan and Hood 2008), allows for a two-year comparison of beetle population growth rate. Both studies were preformed over 7 months and in the same general location. They showed a pattern of adult beetle population increase until the end of July and then a steady decline into fall. The mean number of SHB sampled from the brood chamber is significantly greater than that from the inner cover on all sampling dates except 1 June and 7 September. Greater beetle numbers sampled from the brood chamber is somewhat misleading, as the surface area counted in the brood chamber is 2970 cm^2 and that of the inner cover is 2070.5 cm^2.

Colonies showed a normal build-up of brood throughout the summer and reduction of brood in fall and winter. As colony strength increased, beetle numbers also increased as observed from this study and a previous investigation (Nolan and Hood 2008). While SHB population increased over the summer months, only two test colonies died during the study, however, the minimum colony losses could not be conclusively linked to SHB pressure. Trapping in both the bottom and top of the colonies may have contributed to the high survival rate (92%).

Apiary location was considered as a possible variable in beetle numbers and colony strength. Care was taken to select locations with similar sun and shade; however, other factors not realized might have played a role in both beetle and honey bee colony

survivorship. Regardless of the unrealized differences in apiary location, the results show that beetle numbers and colony strength were similar in all five apiaries. This result is based on similar sun and shade exposure, similar colony strength measured in 25cm^2 brood units, and similar existing beetle populations. Individual colonies did have different beetle numbers; however, the mechanism by which beetles "choose" one colony over another still needs to be investigated.

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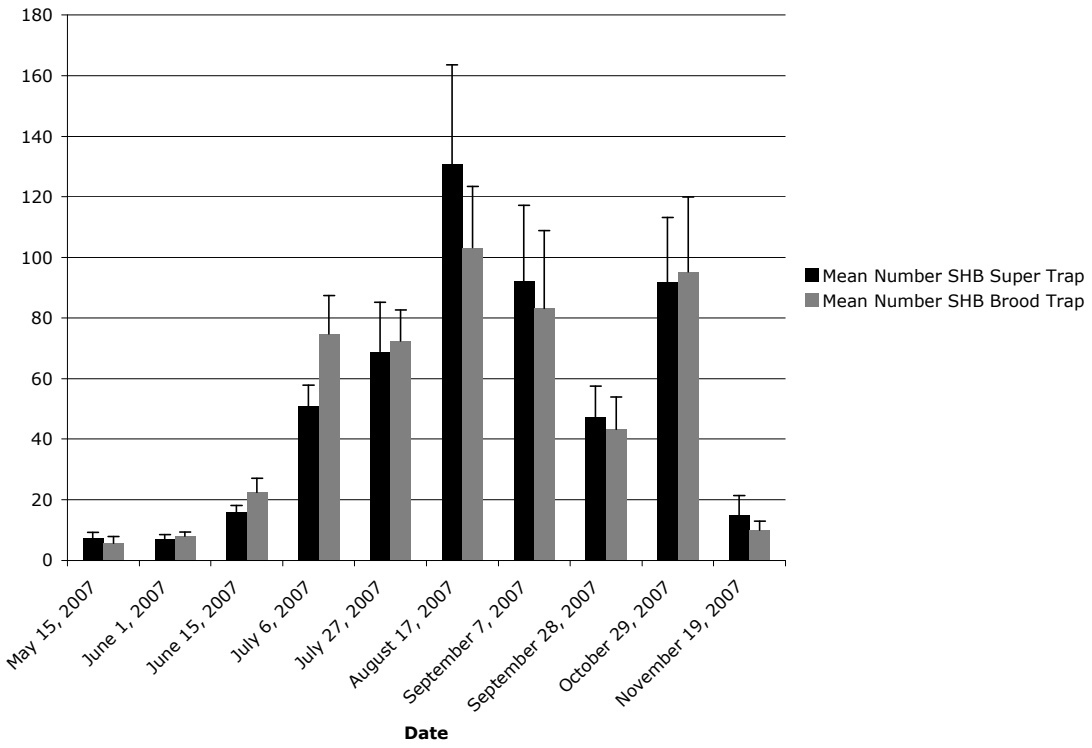

Figure 3.1: Mean number of SHB adults caught in Hood beetle traps during 10 sampling periods in Oconee and Pickens County, South Carolina, May-November 2007. No significant differences where found over the 7-month trapping investigation (P>0.05).

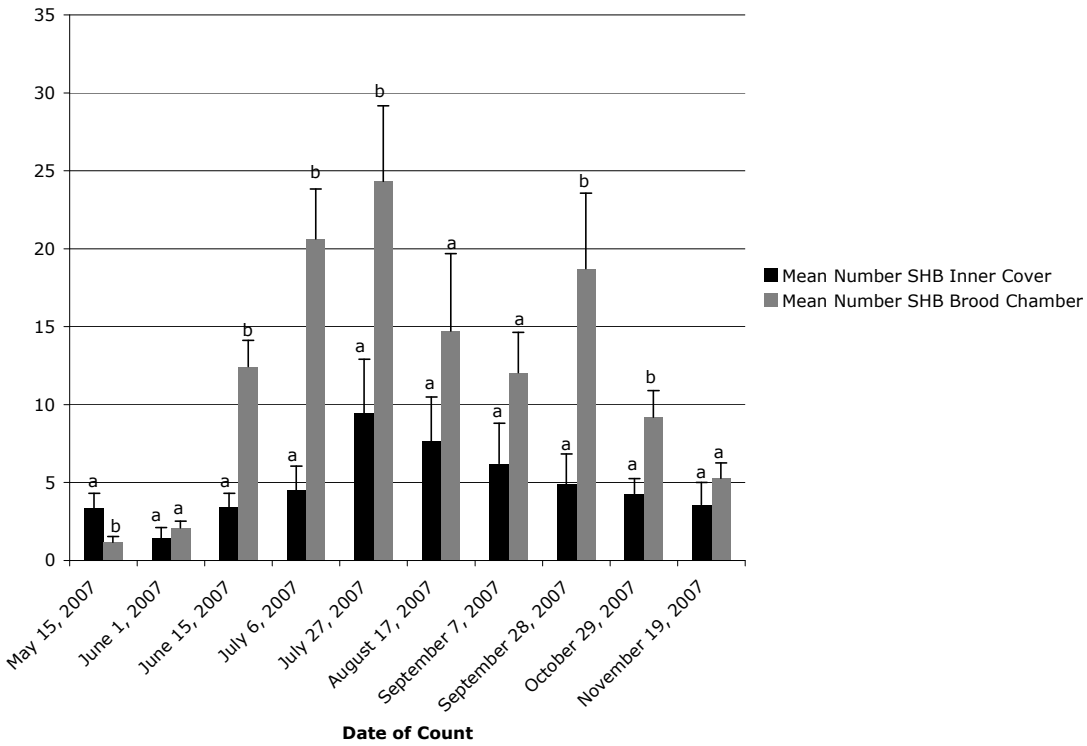

Figure 3.2: Mean number of SHB adults from inner cover and brood chamber over 10 sampling periods in Oconee and Pickens County, South Carolina, May-November 2007. Significant differences ($P<0.05$), observed on all dates excluding 1 June, 17 August, 7 September and 19 November, means followed by the same letter are not different.

CHAPTER 4

Summary

Upon careful consideration of both the methods utilized and the results discerned in my research, several aspects have been identified for further exploration and study. The examination of alternate trapping methods and various applications of the attractants, in addition to further research regarding the relative costs of SHB attractants, could offer significant insight to SHB control.

One potential avenue of study would be monitoring SHB trapping numbers using USDA attractant versus other attractants in the top honey super. A study using only top traps would be insightful, as top-super exclusive trapping has not been investigated. A similar study could take the idea a step further by combining the two attractants with the two trapping locations (top versus bottom) in various combinations. The information gleaned from these investigations may reveal a new trapping location/attractant combination for improved SHB control.

In my research completed regarding the use of the Hood trap with both attractants, mineral oil was utilized only with the attractants, not with the control. The lack of a killing agent in the control prevented the calculation of the number of beetles trapped exclusively by the Hood trap with no attractant. In other words, it was impossible to ascertain the effectiveness of the Hood trap alone. Additional studies utilizing the Hood trap in addition to a killing agent in the control may provide a more thorough comparison of attractant effectiveness. Further, studies comparing the effectiveness of cider vinegar

and the USDA attractant used in various traps should be explored for an improved trap/attractant combination.

An avenue not investigated by this research that needs to be addressed is the cost of various trapping techniques, relative to money and time. Expense is a limiting factor to many beekeepers and in order to get commercial beekeepers to adopt an IPM technique, the economic aspect must be considered. A study of the expense involved in utilizing apple cider vinegar versus the USDA attractant would have practical applications. Placing the relative costs of the attractants into the context of their utilization would assist beekeepers in choosing the most appropriate trapping method. The amount of time spent by beekeepers implementing each control technique could be evaluated in order to determine the cost of labor.

An IPM recommendation, regardless of the technique used, must prove to be as good or better than the current techniques in order for beekeepers to consider change. While pesticidal control has a place as one type of control technique for SHB, other avenues must be explored. These methods should not only be as effective as pesticidal control methods used now, but should be comparable in labor and price. A successful small hive beetle IPM program should include trapping as well as other methods of control such as cultural and biological techniques.