5-2013

# Studies on the Structure and Pathogens of the Small Hive Beetle (Coleoptera: Nitidulidae)

Natasha A. Wright
*University of Arkansas, Fayetteville*

# STUDIES ON THE STRUCTURE AND PATHOGENS OF THE SMALL HIVE BEETLE (COLEOPTERA: NITIDULIDAE)

# STUDIES ON THE STRUCTURE AND PATHOGENS OF THE SMALL HIVE BEETLE (COLEOPTERA: NITIDULIDAE)

A thesis submitted in partial fulfillment
of the requirements for the degree of
Master of Science in Entomology

By

Natasha Anh-Dao Wright
University of Florida
Bachelor of Science in Entomology, 2009

May 2013
University of Arkansas

**Abstract.**

The small hive beetle (*Aethina tumida*, SHB) is an invasive pest of honey bee (*Apis mellifera*) colonies in the United States. The adult and larval beetles can ruin honey through fecal contamination and by vectoring a mutualistic yeast (*Kodamaea ohmeri*) that causes honey fermentation. These beetles also impact honey bee colonies by feeding on bee eggs, bee brood, and pollen. Severe beetle infestations can cause colonies to decline or abscond.

The SHB has been present in the United States since at least 1998. Since then, there have been several published papers on how to successfully rear these beetles. Laboratory rearing of SHBs allows for immediate access to adults and immature stages without having to constantly collect them from infested bee colonies. A clean and cost effective method for rearing SHBs is presented in this thesis.

There is little published information on the external morphology of the SHB. Murray, Schmolke, Menier, and Jouan were some of the few authors to publish on this subject. Photography of the adult and larval stages are provided with emphasis on the adult morphology.

Chemical and cultural controls are typically used to keep SHBs at a tolerable level. Only a few articles have been published on the biological control of SHBs. While some generalist fungal pathogens and commercially available nematodes have been reported to attack SHBs, there have been no reports of any host-specific predators, parasitoids, or pathogenic protozoa, fungi, nematodes, bacteria, or viruses. One protozoan pathogen has been discovered in the process of writing this thesis, but little is known about its life cycle, the effects that it has on SHBs, and whether this pathogen infects other beetles or insects. Dissection techniques for the adults and larvae are discussed in this thesis.

This thesis is approved for recommendation
to the Graduate Council.

Thesis Director:

_____

Dr. Don Steinkraus

Thesis Committee:

_____

Dr. Fred Spiegel

_____

Dr. Ashley Dowling

**Thesis Duplication Release.**

I hereby authorize the University of Arkansas Libraries to duplicate this thesis when needed for research and/or scholarship.

Agreed _____
***Natasha Anh-Dao Wright***

Refused _____
***Natasha Anh-Dao Wright***

**Acknowledgements.**

I give thanks and acknowledgement to my advisor, Dr. Don Steinkraus, and my committee members, Drs. Fred Spiegel and Ashley Dowling.

My research would not have been possible without the aid of Arkansas beekeeping organizations and their beekeepers and bee removers. A special thanks goes out to Jason Ring for his above average willingness to help us obtain infected beetles.

Jon Zawislak deserves not only my acknowledgement but also my respect and deepest appreciation for taking me all over Arkansas to collect beetles and soil samples.

A thanks to Marco Cordero who helped me identify my nematodes to genera.

A thanks to Drs. Jeff Silberman, Harry Kaya, and Jeff Lord for their time and expertise in examining our protozoan pathogen of adult small hive beetles.

**Dedication.**

I thank my family, for without them I would have never made it this far. They have always supported me, and I hope that this thesis will make them proud.

**Table of Contents.**

This thesis consists of the following published papers:

Chapter 5: Wright, N. A., and D. C. Steinkraus. 2012. A scientific note on a protozoan pathogen of the small hive beetle. Apidologie 44(2): 173-175.

**Introduction to the Papers.**

The small hive beetle is a pest of honey bees that has been introduced from South Africa into the United States, Australia, and Canada. In its introduced ranges, this beetle has detrimentally affected honey bees and the beekeepers that care for them. In order for research to be conducted on these beetles, it was necessary to develop a rearing method that would be affordable under a graduate student's limited budget. Once these beetles could be successfully maintained and reproduced, work could be started on examining the beetles for the presence of internal pathogens. This led to the development of dissection techniques for both the adult and larval small hive beetles, followed by an inspection of what pathogenic organisms were found in beetle samples from in and around Arkansas. Not only was there a lack of literature on the internal morphology of the small hive beetle, there was also a shortage of information on the external morphology of this beetle as well. Given that we had the time and the equipment to take high quality photographs, we embarked on a journey to photograph as many external features of the adult beetles as we could. Of course, what the beetles were infected with was one such curiosity, the other being where these beetles were obtaining their infections. Seeing as the small hive beetle spends most of its developmental life in the soil, it was decided that the soil should be examined for pathogenic organisms as well, and many amiable beekeepers allowed me to visit and take of their soil in order to conduct soil bioassays.

## Chapter 1: An Overview of the Small Hive Beetle

### History.

The small hive beetle (SHB), *Aethina tumida* Murray, was first collected about 140 years ago on the west central coast of Africa (Murray 1867). Two specimens were collected from Old Calabar (Nigeria) and sent to Andrew Murray in London, England, for identification (Murray 1867). Although Murray provided an accurate physical description of the beetle, he made no mention of its preferred habitat in honey bee colonies. In fact, Murray provided no information about the habitat from which the two beetle specimens were collected.

It took more than 70 years from the beetle's first collection before anyone began to seriously investigate the life cycle and damage potential of the small hive beetle. Dr. A. E. Lundie, a research apiculturalist at the South African Government Division of Agriculture and Forestry, was the first person to research and write extensively about the small hive beetle. He explained that this beetle should be called the "small hive beetle" in order to distinguish it from a larger beetle, *Hyplostoma fuligineus*, which is also present in honey bee colonies in South Africa (Lundie 1940). Lundie believed that the dearth of information relating to the small hive beetle was due to its misidentification with wax moth larvae, another honey bee pest that is often found in conjunction with small hive beetles (Lundie 1940). Small hive beetles are relatively minor pests of honey bees in Africa, which might explain the lack of early research (Lundie 1940). Lundie's preliminary studies on the biology, longevity, diet, and control of small hive beetles laid the foundation for future research.

More than 30 years after Lundie's work, M. D. Schmolke investigated the small hive beetle further, continuing in Lundie's footsteps. While Schmolke quoted Lundie's previous work, he also realized that some of Lundie's research needed to be redone. For example, Lundie

conducted rearing experiments but did not mention temperature at all. As with most insects, small hive beetle development is highly dependent upon temperature. Schmolke conducted similar rearing experiments in a constant temperature room. He elaborated on Lundie's work and additionally developed sexing techniques, observed beetle and bee interactions in a glass-walled hive, experimented with a variety of soil types on pupation success, and fumigated an entire colony in order to determine where small hive beetles are typically found in a hive (Schmolke 1974).

**General Description of Small Hive Beetle.**

*The Family Nitidulidae.*

Small hive beetles belong to the family Nitidulidae whose members are commonly referred to as sap beetles (White 1983). Members of this family are primarily mycophagous and saprophagous; but phytophagy, necrophagy, and predation are not uncommon (White 1983, Arbogast et al. 2009b). Nitidulids can be separated from other coleopteran families by the following key characters: a three-segmented ball-like antennal club, elongate robust or broadly oval form, strongly transverse front coxae, most with non-striate elytra, one to three abdominal segments are usually exposed beyond the length of the elytra (White 1983). Most nitidulids have five tarsi on all legs with the first three tarsomeres being more or less dilated with the fourth one being very small (White 1983). The fifth tarsomere is elongate and bears two tarsal claws.

A few other nitidulids are also economically important pests. Dried fruit beetles (*Carpophilus hemipterus*) are pests of figs and dates. Although they cause relatively little feeding damage, their feces can spoil fruit along with the fungi they carry (White 1983). Dusky sap beetles (*C. lugubris*) are pests of corn and are often found in conjunction with corn earworms (White 1983).

*Nitidulids in Honey Bee Colonies.*

Besides small hive beetles, other nitidulids have also been found in honey bee colonies (Ellis et al. 2008). Most of these beetles are usually found in colonies infested with small hive beetles (Ellis et al. 2008). *Cychramus luteus* was discovered in honey bee colonies in Germany (Neumann and Ritter 2004). These beetles were not found reproducing in colonies and there was no visible damage to the combs or honey (Neumann and Ritter 2004). *Glischrochilus fasciatus*, a sap beetle which superficially resembles a pleasing fungus beetle, was found in honey bee colonies in Georgia, USA (Ellis et al. 2008). These beetles were mainly found among the colony debris but could also found in leaf litter near colony entrances (Ellis et al. 2008). Only bee colonies near forested areas had *G. fasciatus* (Ellis et al. 2008). No *G. fasciatus* larvae were detected in any of the hives and attempts at rearing the adults in captivity on hive products failed to produce any eggs or larvae (Ellis et al. 2008). Another sap beetle, *Lobiopa insularis*, has also been found among colony debris (Ellis et al. 2008). It has been found feeding on a wide variety of foods, such as fermenting substrates, sap flows, and flowers (Annonaceae: *Annona*) (Ellis et al. 2008). *Epuraea corticina*, typically a feeder on flowers and sap flows, was found on pollen patties (Ellis et al. 2008). These nitidulids appear to be mainly mycophagous or saprophagous and are not likely to cause any major problems for honey bees (Ellis et al. 2008). Because most of these beetles seemed to occur in small hive beetle-infested hives, it is possible that they were attracted to the fermenting hive products (Torto et al. 2007a, Ellis et al. 2008).

*Description of Small Hive Beetle Life Stages.*

Small hive beetle eggs are about two-thirds the length of honey bee eggs (Fig. 1). Lundie (1940) reported small hive beetle eggs as being 1.4 mm long and 0.26 mm wide. The eggs are bright white and oviposited in clusters in areas hidden from worker bees (Lundie 1940,

Schmolke 1974). Larvae emerge through a slit at the front end of the egg (Lundie 1940). The larvae are covered in a number of short spines and protuberances (Fig. 2). Mature larvae range from 8.6 to 10.5 mm in length (Fig. 3) (Schmolke 1974). The pupae have thin, flexible projections on their thorax and abdomen. Young pupae are completely white (Fig. 4) (Lundie 1940). As the pupae mature, the hind wings darken considerably (Fig. 5) (Lundie 1940).

Upon eclosion, the adults are a light yellowish brown color (Fig. 6). Once their exoskeleton has fully sclerotized, older adults may appear to be dark brown or even black (Fig. 7) (Lundie 1940). The adult beetles are broadly oval and convex (Murray 1867). Their shape is defensive in purpose, as their dome-like shape allows them to hide their head and extremities from the aggressive actions of honey bee workers (Schmolke 1974). The lateral margins of the prothorax, elytra, and exposed abdominal segments are covered in yellow-brown setae (Murray 1867). A thick layer of yellow-brown setae can be found on the underside of the beetles as well (Murray 1867). The legs are broad and flat (Murray 1867). The femora are grooved, allowing for the tibiae to fold in (Murray 1867).

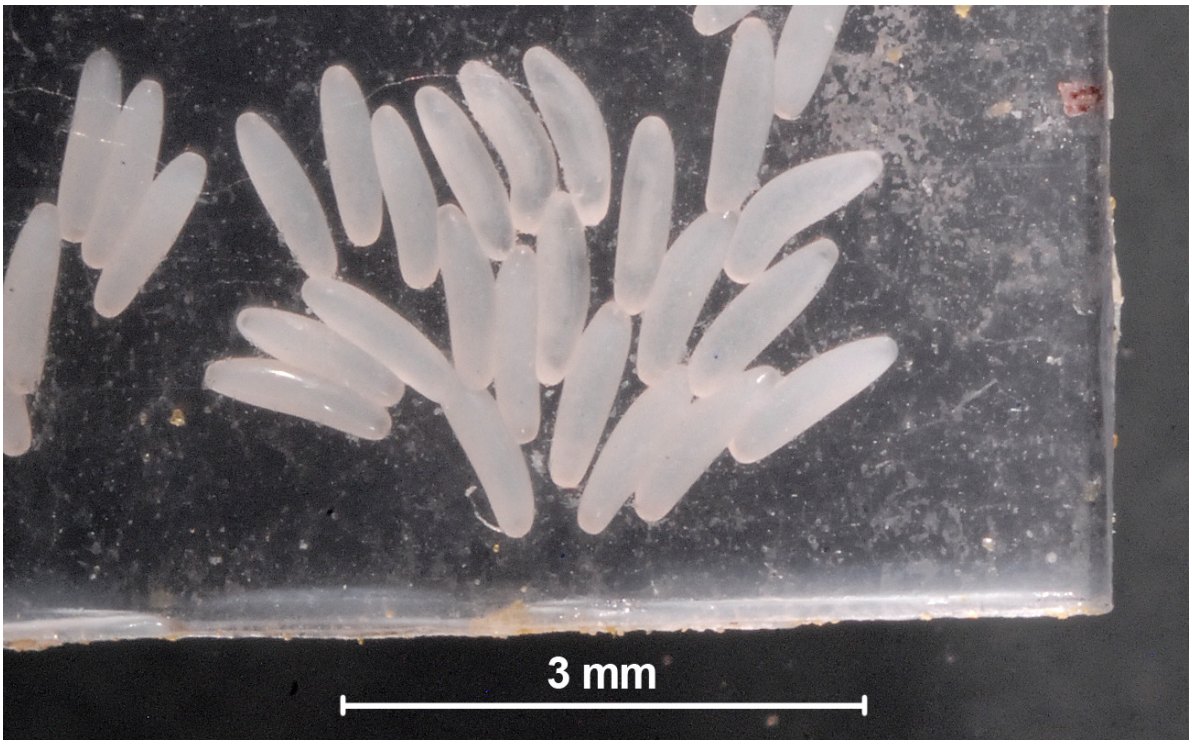


Fig. 1. SHB eggs in a plastic oviposition strip.

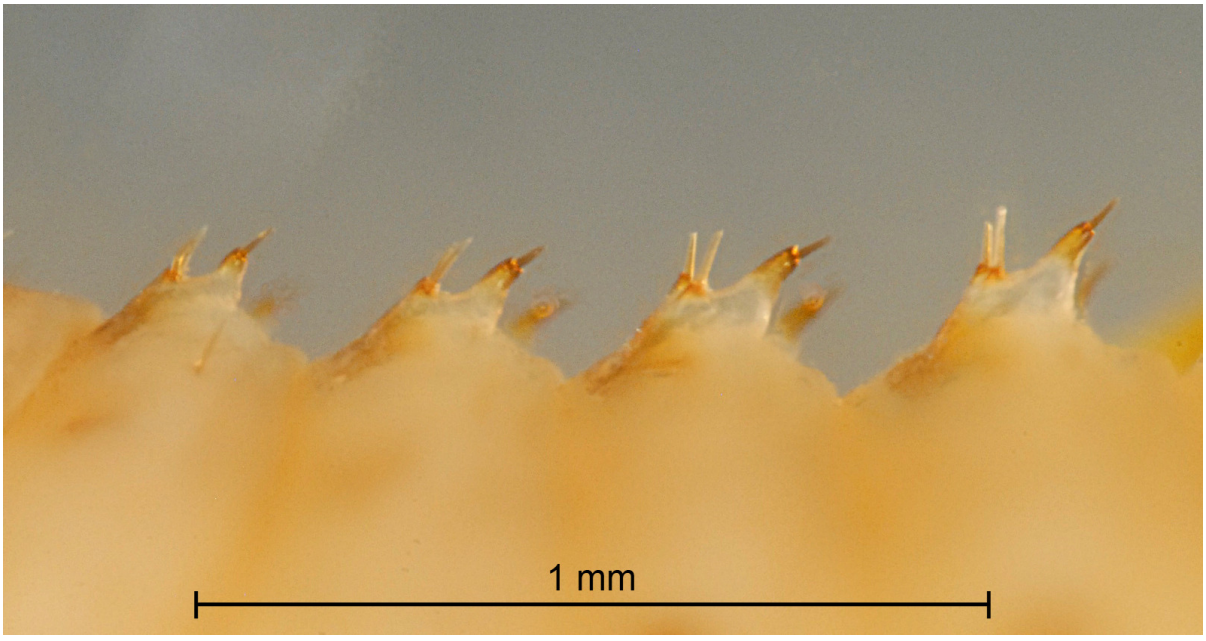


Fig. 2. Dorsal protuberances of 3rd instar SHB. The anterior end is to the left.


Fig. 3. Third instar larva.

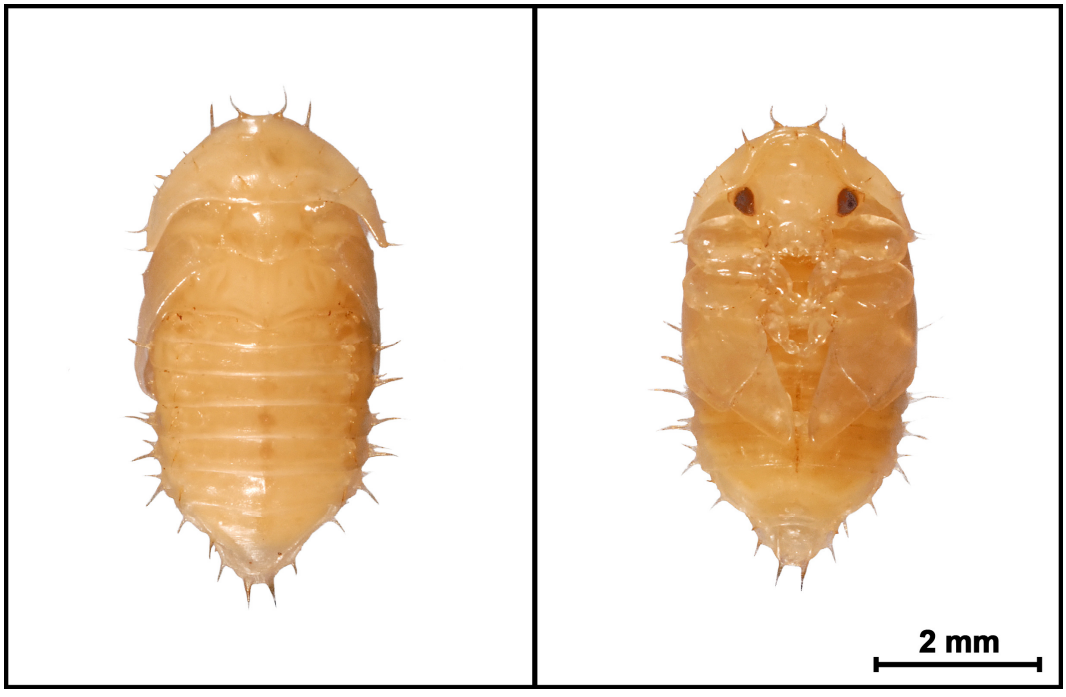


Fig 4. Young pupa.

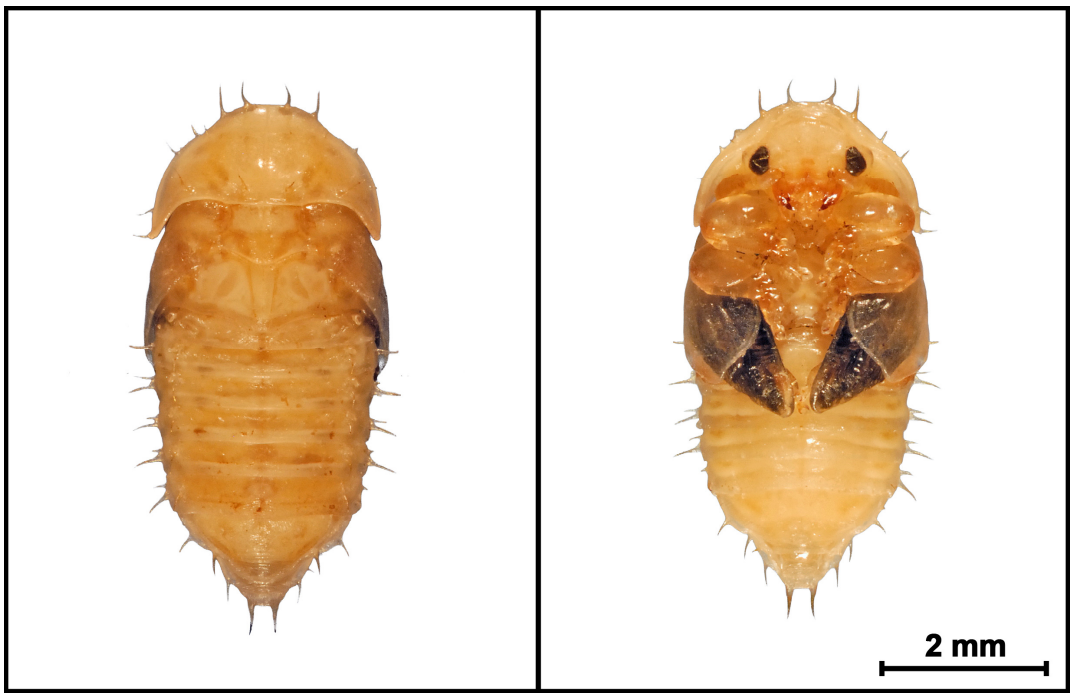


Fig. 5. Mature pupa.

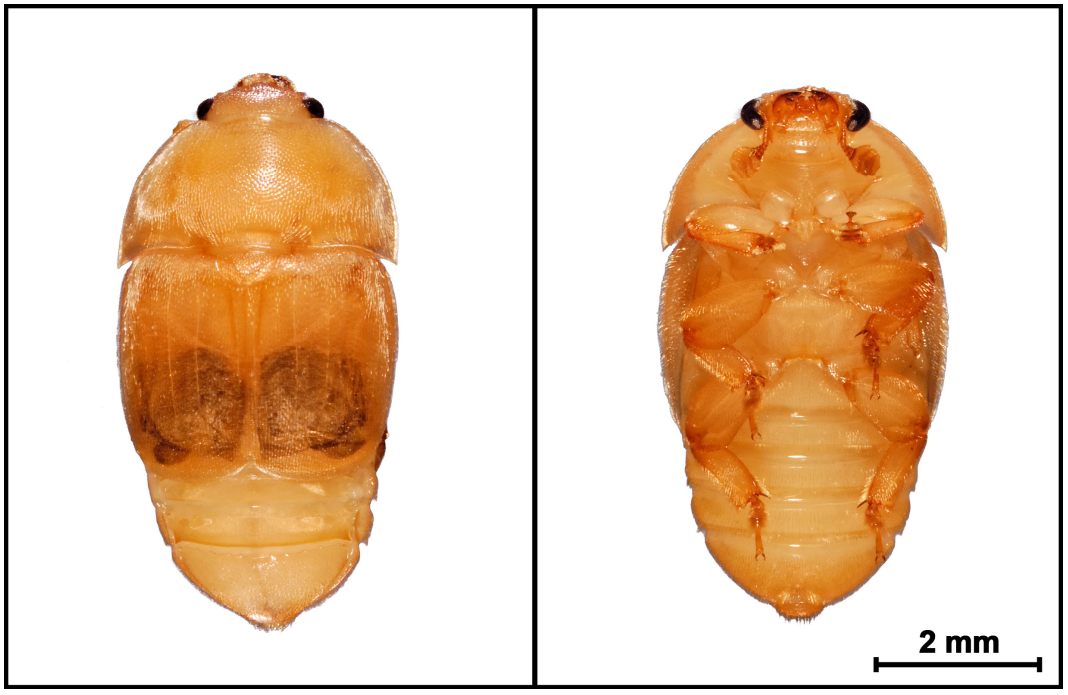


Fig. 6. Adult immediately after eclosion.

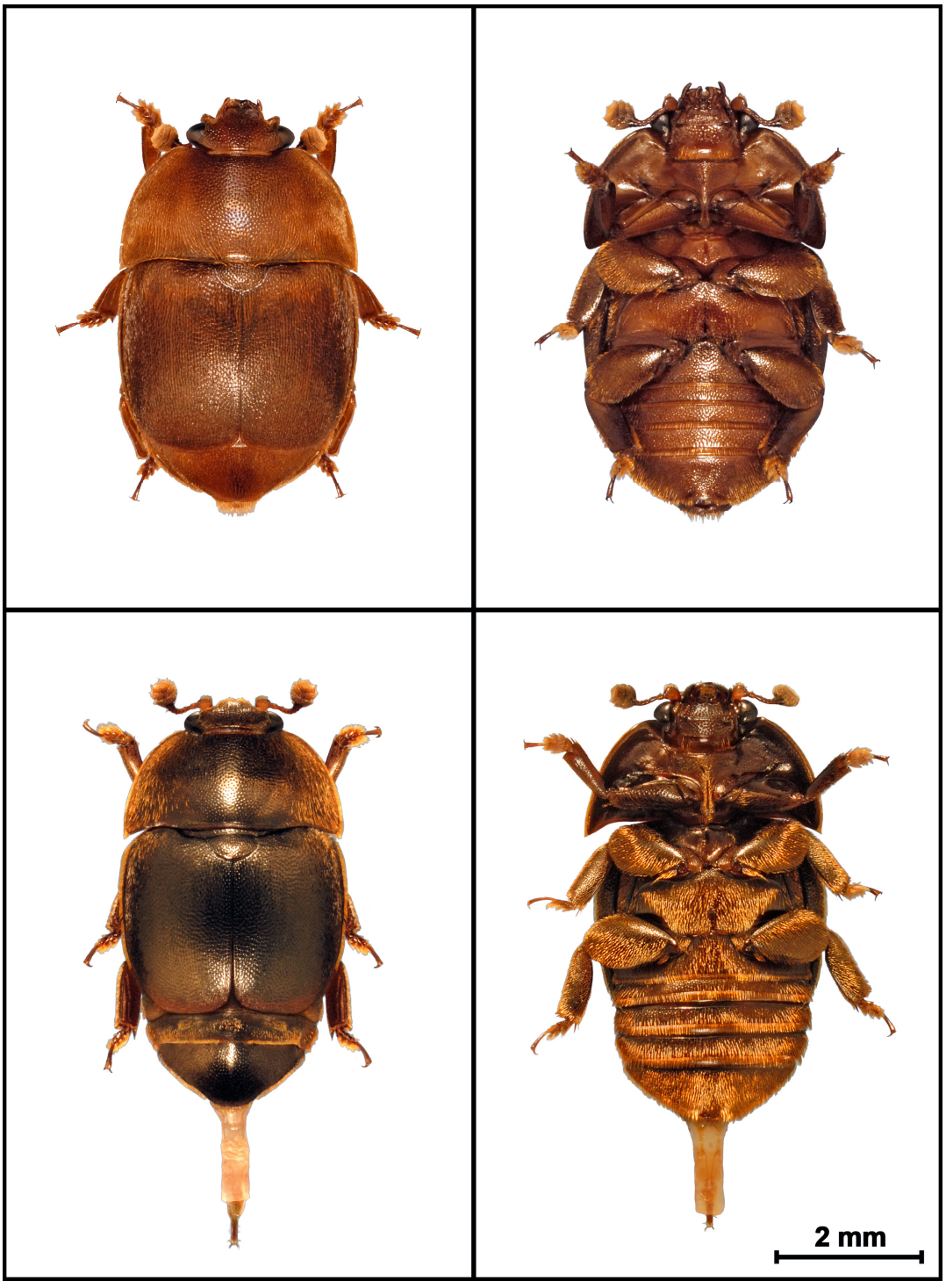


Fig 7. Mature adult beetles. Top row, male. Bottom row, female.

While examining field-collected specimens in Africa, Schmolke (1974) noted that male beetles were shorter in length and width than female beetles. Ellis et al. (2002a) found similar results across three locations in the southeastern United States. For example, male beetles from Clemson, South Carolina were smaller (5.6 ± 0.04 mm) than female beetles (5.8 ± 0.04 mm) from the same location (Ellis et al. 2002a). The width of male beetles (3.2 ± 0.03 mm) did not differ significantly from female beetles (3.2 ± 0.02 mm) (Ellis et al. 2002a). Female beetles also weighed significantly more (13.2 ± 0.03 mg) than male beetles (11.7 ± 0.3 mg) (Ellis et al. 2002a). Most studies reported that females outnumbered males in most samples, showing a female biased operational sex ratio (Ellis et al. 2002a, Neumann et al. 2001, Mürrle and Neumann 2004, Cuthbertson et al. 2008). However, Torto et al. (2009b) observed equal numbers of females and males when beetles were raised on a variety of diets. Schmolke (1974) also found a 1:1 ratio when sampling all of the beetles from two hives and also when raising them at 30°C. Depending upon larval maturation time and nutrition, the sizes of adult female and male beetles can overlap and, therefore, should not be used as an indicator of sex (Schmolke 1974). The proper way to sex beetles is to hold the beetle near the middle with a pair of forceps (Schmolke 1974). When firm dorsal/ventral pressure is applied, females will extend their ovipositor and males will extend their 8$^{th}$ tergite (Schmolke 1974).

**Occurrence and Spread.**

*Occurrence in Native Range.*

The small hive beetle is indigenous to sub-Saharan Africa and occurs throughout many tropical and subtropical areas (Lundie 1940). As of 2008, the following 24 African countries were known to have small hive beetles: Angola, Botswana, Cameroon, Central African Republic, Congo Republic, Democratic Republic of Congo, Ethiopia, Eritrea, Ghana, Guinea Bissau,

Kenya, Lesotho, Malawi, Mozambique, Nigeria, Namibia, Senegal, South Africa, Southern Sudan, Swaziland, Tanzania, Uganda, Zambia, and Zimbabwe (Mostafa and Williams 2002, Ellis 2004, Hood 2004, Neumann and Elzen 2004, Neumann and Ellis 2008). The true native range of this beetle probably overlaps with that of African honey bees (Ellis 2004). While beetles were discovered in Egypt in 2002, no established populations could be found during surveys conducted in 2008 (Hassan and Neumann 2008, El-Niweiri et al. 2008).

*Occurrence in Introduced Range.*

In 1998, small hive beetles were collected from honey bee colonies around St. Lucie, Florida (Elzen et al. 1999b, Hood 2004). These specimens were sent to and identified by Dr. Michael C. Thomas of the Florida Department of Agriculture in Gainesville, Florida (Elzen et al. 1999b, Hood 2004). While these beetles were the first to be reported in the United States, there were apparently unidentified beetle collections that suggest small hive beetles were present since at least November of 1996 from Charleston, South Carolina (Hood 2004). It is likely that the small hive beetle arrived in Charleston port aboard cargo ships from Africa (Hood 2004). From Charleston, the beetles spread throughout South Carolina, Georgia, Florida, and also a single county in North Carolina by 1998 (Hood 2004). By 1999, these beetles were found in nine additional states (Hood 2004). While the small hive beetle has probably spread slightly due to natural range expansion, the alarming rate at which it has been spreading across the United States is no doubt due to the movements of infested colonies, package bees, beeswax, and beekeeping equipment (Hood 2004). As of 2008, the spread of the small hive beetle has encompassed at least 31 states in total: Alabama, Arkansas, California, Delaware, Florida, Georgia, Illinois, Indiana, Iowa, Kentucky, Louisiana, Maine, Maryland, Massachusetts, Michigan, Minnesota, Mississippi, Missouri, New Jersey, New York, North Carolina, North Dakota, Ohio, Pennsylvania, South

Carolina, Tennessee, Texas, Vermont, Virginia, West Virginia, and Wisconsin (Hood 2004, Neumann and Elzen 2004, Neumann and Ellis 2008). However, small hive beetles tend to cause more damage throughout Florida and along the coastlines of Georgia, South Carolina, and North Carolina than in other regions of the U.S. (Neumann and Elzen 2004). The sandy soils, high humidity, warm temperatures, and regular rainfall may contribute to its more severe pest status in these areas (Somerville 2003).

Mitochondrial DNA evidence suggests that small hive beetles in the U.S. originally came from South Africa (Evans et al. 2000, 2003). These beetles differed in mtDNA haplotypes by 0.4%, an acceptable variation that can be found among populations across South Africa (Evans et al. 2000). When using the mitochondrial cytochrome oxidase I gene, beetles from the U.S. were practically identical to beetles from South Africa and Zimbabwe (Evans et al. 2003, Hood 2004). Beetles from the U.S. did exhibit significantly lower haplotypic diversity than beetles from South Africa; this may indicate limited introductions or multiple population bottlenecks (Evans et al. 2000, Hood 2004). While this does not provide conclusive evidence as to where U.S. populations of small hive beetles came from, it is highly likely these beetles invaded from South Africa (Evans et al. 2003).

In June of 2000, small hive beetles were detected in Egypt in Etaie-Al-Baroud just northwest of Cairo (Mostafa and Williams 2002); however, a recent survey shows that small hive beetles are not well established in Egypt (Hassan and Neumann 2008). A 2008 survey found no evidence of beetles in colonies throughout Egypt (Hassan and Neumann 2008). A total of 1239 local colonies were inspected in 11 districts throughout Egypt (Hassan and Neumann 2008). Soon after the survey, small hive beetles were found in Sudan in Southern Darfur State (El-Niweiri et al. 2008). However, only two out of 25 colonies had beetles, and both infested

colonies had less than 10 adult beetles each (El-Niweiri et al. 2008). No beetles were found in the central or northern states, including the northernmost state that borders Egypt (El-Niweiri et al. 2008). The lack of beetles in Egypt and the northern part of Sudan is likely due to very dry conditions and solid rock substrates, both of which may hinder successful pupation (El-Niweiri et al. 2008). Based on these two studies (Hassan and Neumann 2008, El-Niweiri et al. 2008), it appears that the native range of the small hive beetle is restricted to sub-Saharan Africa. While the natural northernmost range of the beetle appears to be in Sudan, beetle presence in Egypt is probably due to introduction (Hassan and Neumann 2008, El-Niweiri et al. 2008).

In August of 2002, small hive beetle larvae were detected in Manitoba, Canada (Dixon and Lafrenière 2002). The larvae were transported via a shipment of infested wax cappings from Texas to a Canadian wax rendering facility (Dixon and Lafrenière 2002, Hood 2004). Inspections revealed one adult beetle in a nearby honey bee pollen trap (Dixon and Lafrenière 2002). In an effort to prevent an infestation in Manitoba, the soil around the facility was treated with insecticides in hopes of killing any larvae or pupae that may have escaped the building (Dixon and Lafrenière 2002). This appeared to be an isolated incident as no other detections could be made around the Manitoba area after conducting extensive surveys (Dixon and Lafrenière 2002, Hood 2004). In 2006, adult beetles were found in colonies in Alberta and Manitoba (Lafrenière 2007). This second introduction was also successfully eradicated (Lafrenière 2007). In 2008, a third introduction was found in Quebec near the U.S. border (Evans 2010). In April of 2011, adults were found overwintering in a wrapped colony in Quebec, confirming the fears that these beetles are able to survive the cold temperatures of Canada (Evans 2011). In March of 2011, several apiaries in the province of Ontario were found to have beetles and were placed under quarantine (Evans 2011). Despite Canada's efforts in preventing

the spread of the small hive beetle, it appears that this beetle has finally gained a foothold in Canada. The beetles most likely arrived from infested, bordering U.S. states (Evans 2011).

In October of 2002, small hive beetles were detected in Australia (Fletcher and Cook 2002, Hood 2004). A beetle-infested colony was discovered in Richmond, northwest of Sydney, New South Wales (Fletcher and Cook 2002). New South Wales Agriculture coordinated a statewide response following the declaration of an exotic 'disease' outbreak (Gillespie et al. 2003). Commercial and feral hives within 3 km of the detection site were inspected for signs of small hive beetles (Gillespie et al. 2003). Beekeepers were also mailed a survey to check their own hives for the presence of the beetle (Gillespie et al. 2003). There were a total of 120 positive detections with 12 of those being found in feral colonies (Gillespie et al. 2003). Small hive beetles appeared to be concentrated in the Sydney basin and also at Cowra, Binalong, and the Stroud in the Hunter Valley (Gillespie et al. 2003). Although it is unknown how long small hive beetles had been present in Australia, it appears that the beetles were brought into the Sydney basin and transported to other areas through the movement of infested hives (Gillespie et al. 2003). After the study by Gillespie et al. (2003), beetles were detected in Queensland (Anonymous 2003). It appears that infested hive material was sent from Sydney to an apiary in Beerwah, resulting in beetle populations in Queensland (Anonymous 2003). Reports indicate that small hive beetles cause significantly less damage in Australia than in the United States (White 2003). The climate of Australia may be less suitable for beetle reproduction and development (Somerville 2003). Drought conditions may slow small hive beetle movement to other un-infested Australian regions (Somerville 2002).

In April of 2010, the small hive beetle was discovered in Hilo, Hawai'i (State of Hawai'i, Department of Agriculture 2010). The introduction of this new hive pest may have devastating

effects on the queen bee production and exportation of Hawai'i (Connor 2011). The route of invasion is not known. A Pana'ewa beekeeper reported the beetles to an entomologist at the Hawai'i Department of Agriculture (State of Hawai'i, Department of Agriculture 2010). Neil Reimer of the Plant Pest Control Branch stated that "the small hive beetle will be difficult to eradicate and control because it also feeds on various decaying fruits which are abundant in the wild" (State of Hawai'i, Department of Agriculture 2010). However, it is not known how efficient these beetles are at utilizing other resources for food, as they may be outcompeted by more prolific or specialized scavengers and opportunists (Buchholz et al. 2008). In a survey of 2010 honey bee colony losses in Hawaii, 80% of respondents reported losing colonies due to small hive beetles and 29% of respondents reported losing colonies to small hive beetles and *Varroa* mites (Connor 2011). Ninety percent of respondents lost a colony during 2010, and 34% had no colonies remaining at the end of the year (Connor 2011).

*Potential Invasion Areas.*

The small hive beetle is not present in Europe (Cuthbertson et al. 2008). Great care is being taken to prevent this beetle from invading the United Kingdom, since the climatic conditions there would be suitable for small hive beetle survival and reproduction (Brown et al. 2002, Cuthbertson et al. 2008). Small hive beetles may gain entry via the importation of package bees or cage queens (Brown et al. 2002). However, the importation of fruits should also be considered as a potential invasion route (Brown et al. 2002). Although less likely, the movement of soil may unknowingly spread small hive beetle larvae or pupae to the UK (Brown et al. 2002). This beetle may also be a threat to the UK's native bumble bee species (Brown et al. 2002). Beetles imported into the UK for research purposes are kept secure under three layers of containment to prevent escapes en route to their destination. Cages containing the beetles are

kept in a sealed room which could only be accessed via a freezing corridor (-15°C) (Cuthbertson et al. 2008).

The small hive beetle has not been found in Asia. Since Asia has a high diversity of bees (including nine indigenous *Apis* species), it is imperative that all steps be taken to prevent this beetle from invading Asia (Ellis 2004, Oldroyd and Nanork 2009). These indigenous honey bees pollinate about 33% of crop species in Asia and several bird species feed exclusively on these bees (Oldroyd and Nanork 2009). Should the small hive beetle be introduced, it is possible that the beetle could host switch to other bee species or bee genera. Studies have shown that the small hive beetle is able to reproduce in bumble bee colonies (Ambrose et al. 2000, Spiewok and Neumann 2006b, Hoffmann et al. 2008) and potentially in stingless bee colonies (Greco et al. 2010), both of which are in different genera from their native host. Deforestation and honey hunting already negatively impact Asian honey bees; the introduction of a novel colony parasite may cause these species to decline even faster (Oldroyd and Nanork 2009).

*Spread.*

Migratory beekeeping practices have contributed greatly to the spread of small hive beetles (Hood 2000, Caron et al. 2001, Wenning 2001). A migratory beekeeper introduced small hive beetles to Maryland from Florida in April 2001 (Caron et al. 2001). Migratory colonies from New Jersey that had visited Florida found beetles in their colonies after returning home (Caron et al. 2001). These beetles also plague the honey houses of these migratory beekeepers (Caron et al. 2001). Unfortunately, migratory beekeepers are not the only ones troubled by this beetle. Non-migratory beekeepers located near migratory beekeepers have reported seeing small hive beetles in their colonies (Caron et al. 2001). There are undoubtedly many more unreported cases of migratory colonies transporting small hive beetles to un-infested areas.

Small hive beetles are also able to be spread through package bees and possibly queen cages (Caron et al. 2001, Brown et al. 2002, Ellis 2004). In 1999, beetles were found in packages sent from South Carolina to New Jersey (Caron et al. 2001). Apiaries that received infested packages were quarantined and treated with coumaphos (Caron et al. 2001). Follow up inspections found no beetles (Caron et al. 2001). In 2001, small hive beetles were found in package bees sent from Georgia to Delaware (Caron et al. 2001). One beetle was found on the outside of a package and captured by a graduate student at the University of Delaware (Caron et al. 2001). Another adult was found a week later at a sugar-water feeder (Caron et al. 2001). Two weeks later during a routine inspection of the hive, one more adult was discovered (Caron et al. 2001). All adults were captured and removed, and three months later no small hive beetles could be found (Caron et al. 2001). Another infested package from the same Delaware shipment was also sent to Maryland (Caron et al. 2001). Two weeks after the package had been installed, three live and three dead adult beetles were found in the colony (Caron et al. 2001). Two weeks later, nearly 30 wandering larvae were found on the bottom board screen (Caron et al. 2001). The ability of small hive beetles to spread via packages has had negative effects on the queen and package bee industry (Ellis 2004). For example, Canada refused to import queens from Australia because of the risk of small hive beetle introduction (T. Weatherhead, personal communication in Ellis 2004).

**Damage.**

*Damage in Africa.*

The small hive beetle causes only minor problems in its native range to its native hosts, *Apis mellifera scutellata* and *A. m. capensis*. It is a mainly a nuisance of honey houses, a destroyer of weak or diseased colonies, and a scavenger of colonies left by absconding bees

(Schmolke 1974, Elzen et al. 1999b, Ellis et al. 2003b, Ellis 2004, Hood 2004). The beetle is oftentimes found in abandoned hives or in stored honey combs, usually in the absence of honey bee workers (Lundie 1940, Schmolke 1974). This beetle rarely requires control in Africa (Elzen et al. 1999b). Inspection of South African hives revealed low numbers of adult beetles (an average of 20 beetles per hive) and almost no larvae (Elzen et al. 2000a). Reproduction is limited in their native host hives, as African honey bees are efficient at controlling their numbers (Ellis 2004). Successful reproduction mainly occurs in abandoned hives where food stores are unprotected (Ellis 2004). Few behavioral differences were seen between beetles in Africa and beetles in the United States (Elzen et al. 2000a). Because small hive beetles are relatively minor honey bee pests in Africa, little work was done on them until 1940. Their introduction into other parts of the world has expedited research on all aspects of this bee colony pest.

*Damage in the United States.*

In their introduced range, small hive beetles are considerably more destructive. The honey bees that are affected are mainly *A. m. ligustica*, *A. m. carnica*, and *A. m. caucasica* (Pettis and Shimanuki 2000). A single colony can house more than 1,000 adult beetles (compare to about 20 beetles per hive for African colonies) (Elzen et al. 1999a, 2000). Somerville (2003) stated that a single frame could contain upwards of 6,000 larvae. These large beetle infestations can cause the rapid decline or collapse of European honey bee colonies, seemingly regardless of colony strength (Elzen et al. 1999b, Hood 2004). In severe cases, these beetles can overrun an entire apiary and cause multiple colony collapses (Hood 2000). In 1998, small hive beetles caused approximately US$3 million dollars in damage in the U.S., destroying over 30,000 colonies (Hood 2004, Neumann and Elzen 2004). The damage estimate also includes ruined comb and honey losses due to fermentation (Hood 2004).

These beetles caused substantial problems during the first few years of their introduction. Colony mortality was high and bees were more likely to abscond when beetles were present (Hood 2000). Before effective control measures were in place, commercial beekeepers lost thousands of colonies as well as beekeeping equipment to uncontrollable small hive beetle infestations (Hood 2000, Somerville 2003). Their increased damage potential in the United States may be attributed to European honey bee behavior, weather and/or soil conditions, and also a possible lack of natural enemies such as viruses, bacteria, fungi, protozoa, or nematodes (Ellis 2004, Hood 2004).

*Increased Beetle Populations in Association with Patties.*

Beekeepers have reported increased beetle infestation in colonies that had patties of any variety, including pollen substitute patties, grease patties for tracheal mites, and oxytetracycline-laden patties for American foulbrood control (Westervelt et al. 2001, Elzen et al. 2002). Westervelt et al. (2001) conducted the first study on patties and their effect on small hive beetles. Grease patties consisting of sugar, vegetable shortening, and antibiotics were placed in colonies with no infestations and in colonies with minor infestations (Westervelt et al. 2001). Untreated hives served as controls. In one week, treated colonies with previously low numbers of beetles suddenly had very high numbers of adult beetles (around 1,000) (Westervelt et al. 2001). Larvae were also found within the patty (Westervelt et al. 2001). Untreated colonies did not exhibit the same jump in their infestation levels (Westervelt et al. 2001). Similar results were found in another study by Elzen et al. (2002) which involved grease patties with Tylosin (an antibiotic that can be utilized against oxytetracycline-resistant American foulbrood). Tylosin was effective when applied in patty form and also as a dust with confectioner's sugar (Elzen et al. 2002). However, Tylosin patties resulted in higher adult beetle populations; within a week it was

apparent that more adults were migrating into patty-treated hives (Elzen et al. 2002). Larval populations also increased, and larvae were observed crawling through the patty (Elzen et al. 2002). Because the dust application is equally as effective, it is recommended over the patty treatment (Elzen et al. 2002). This is also beneficial because the dust does not persist as long as the patty, reducing the amount of time that the antibiotic comes into contact with wax and honey, resulting in less residue accumulation (Elzen et al. 2002).

*Damage to Honey, Pollen, and Brood.*

Both adults and larvae feed on stored honey (Lundie 1940, Schmolke 1974). They defecate in their own food supply, causing the honey to become fouled, discolored, and fermented (Lundie 1940, Schmolke 1974, Torto et al. 2007c). Larvae bore through honeycomb and crawl through tainted honey, leaving slimy trails that discolor everything they crawl across (Lundie 1940). The fermented honey is thin, runny, bubbly, and has a distinctive smell like rotting oranges (Lundie 1940). When infestation levels are high, fermented honey can run out of the cells, drip onto the bottom board, and run out of the hive entrance (Lundie 1940). Contaminated honey is rejected by the bees and should not be marketed or sold for human consumption (Hood 2004). If the honey is allowed to ferment further, it becomes granular and spongy in texture, making it difficult to clean from the hive (Lundie 1940).

In the laboratory environment, small hive beetle adults and larvae preferred bee eggs and brood over both pollen and honey (Schmolke 1974, Elzen et al. 1999a, Neumann et al. 2001a), but it is unlikely that small hive beetles actually cause significant brood decline in a field environment (Ellis et al. 2003b). At moderate infestation levels, honey bees are able to keep most adult beetles away from the brood comb (Lundie 1940, Schmolke 1974, Spiewok et al. 2007).

*Association with Yeasts.*

Hives infested with small hive beetle larvae often have some degree of fermentation (Lundie 1940, Schmolke 1974, Benda et al. 2008). The plating of beetle homogenates (from adult beetles collected from beetle-infested hives) revealed the presence of the yeast *Kodamaea ohmeri* (Benda et al. 2008). *K. ohmeri* appears as smooth, cream-colored colonies when grown on agar dishes (Benda et al. 2008). At very high infestation levels, *K. ohmeri* was the only yeast found in infested honey bee colonies (Benda et al. 2008). Colonies that had no beetles or very few beetles had a broader fungal diversity which included many species of yeasts and other fungi (Benda unpublished in Benda et al. 2008). It appears that *K. ohmeri* can out-compete other yeasts in the hive and prevent them from establishing, although it is unknown exactly how *K. ohmeri* is able to suppress other yeasts from growing (Benda et al. 2008).

When grown on pollen, *K. ohmeri* produces compounds which are also present in honey bee alarm pheromone (Torto et al. 2007a, Benda et al. 2008). These compounds are highly attractive to adult beetles (Torto et al. 2007a). However, when the yeast was grown on media without actual pollen (i.e. pollen substitute), it did not produce the same compounds (Benda et al. 2008). There may be something vital in bee-collected pollen that allows for the production of these attractant volatiles (Benda et al. 2008).

As small hive beetle larval frass increases, so does the fermentation of hive products (Torto et al. 2007c). Torto et al. (2007c) noted that both adult beetles and honey bee workers chose to abandon hives that were severely infested with larvae. The buildup of fermentation products, particularly 2-phenylethanol, was found to reduce the attractiveness of pollen dough in the laboratory (Torto et al. 2007c). Small hive beetle larvae do not seem to be negatively affected even though they are in close contact with fermenting hive products. This may be a dispersal mechanism to prevent beetle overcrowding in colonies. The mobile adults are able to detect these

high levels of fermentation volatiles and disperse to new colonies while the larvae stay in the abandoned hive and develop to maturity.

It is likely that *K. ohmeri* is picked up by adult beetles in infested colonies and transported to un-infested colonies (Benda et al. 2008). However, members of the genus *Kodomaea* have also been detected among certain flowers, so it is possible that honey bee foragers could also be transporting yeast spores to the colony (Torto et al. 2007a).

*Reduced Flight Activity.*

High beetle populations disrupt and lower the flight activity of European colonies (Ellis et al. 2003b). Workers must spend time chasing adult beetles from the brood comb, guarding the beetles in prisons, and removing cell contents with infested brood and beetle eggs (Ellis et al. 2003b). These behaviors take away from the care and construction of the colony. Because foraging bees are in the same age cohort as guarding bees, bees of this age range are capable of doing either of the two tasks (Ellis et al. 2003c). An increase in small hive beetles results in an increase in guard bees, resulting in lower numbers of available foraging workers (Ellis et al. 2003b).

*Winter Losses.*

A study by Schäfer et al. (2010a) showed that small hive beetles do not significantly affect the winter losses of honey bee colonies in Maryland. In their native range, small hive beetles and their African honey bee hosts do not need to overwinter because of warm year-round temperatures (Hepburn and Radloff 1998). In the United States, though, adult beetles have been found overwintering in European colony clusters (Hood 2004). However, it appears that a very small portion of adult beetles overwinter successfully in these winter clusters (Schäfer et al. 2010a). Overwintering bees that were infested with *Varroa destructor* and small hive beetles

suffered high losses, but all groups infested with *V. destructor* had significant losses (Schäfer et al. 2010a).

*Potential Vectors of Honey Bee Diseases.*

The small hive beetle may be a potential vector of honey bee diseases (Eyer et al. 2009a). As scavengers, these beetles may acquire pathogens through feeding on diseased brood, dead worker bees, dead beetles, and contaminated bee products (Eyer et al. 2009a). Trophallaxis with infected workers (behavioral mimicry, discussed later) may also spread bee pathogens to beetles (Eyer et al. 2009a). While very little research has been done, it has been found that small hive beetles can become contaminated with *Paenibacillus larvae* spores which can result in American foulbrood (=AFB) (Schäfer et al. 2010b). They may also become infected with deformed wing virus (=DWV) and honey bee sacbrood virus (=SBV) (Eyer et al. 2009a, 2009b).

Small hive beetles and *P. larvae* are both present in the U.S. but very little information exists on their possible interactions (Ellis and Munn 2005, Schäfer et al. 2010b). Adult beetles and larvae are capable of picking up *P. larvae* spores from contaminated surfaces (Schäfer et al. 2010b). Larvae continued to hold spores through pupation and as newly-emerged adults (Schäfer et al. 2010b). Wandering larvae had the highest spore numbers (>5000 spores per larva), most likely from feeding on contaminated brood and crawling through contaminated wax (Schäfer et al. 2010b). Hansen and Brødsgaard (1997) found that the minimum number of spores required for an AFB outbreak was of the order of 2,000,000,000 spores, a number significantly higher than what several hundred adults could carry into a colony (Schäfer et al. 2010b). However, only very small doses of *P. larvae* spores are needed to kill honey bee larvae (Schäfer et al. 2010b). Only 8.5 spores per larva were required to kill 50% of 24-48 hour old brood while 51.4 spores per larva killed 90% of the same group (Schäfer et al. 2010b). While small hive beetles are only

likely to transmit small numbers of *P. larvae* spores, it is still possible that they could infect young brood (Schäfer et al. 2010b). This could be detrimental to colonies that only have young brood, but clinical outbreaks of AFB due to beetle contamination are less likely (Schäfer et al. 2010b).

Research results show that small hive beetles can become infected with DWV by feeding on dead contaminated workers or contaminated brood (Eyer et al. 2009a). Beetles may also become infected from contaminated wax and through trophallaxis with contaminated workers (Eyer et al. 2009a). Beetles may also become infected by feeding on contaminated pollen, but further research needs to be conducted (Eyer et al. 2009a). Only 40% of beetles were shown to have DWV replication (Eyer et al. 2009a). While there is no information on the actual effect of small hive beetles as vectors of DWV, it may be possible for DWV and other bee viruses to replicate in small hive beetles as another way of gaining entry into bee colonies (Eyer et al. 2009a).

Adult beetles can become infected with SBV by feeding on contaminated bee brood (Eyer et al. 2009b). The virus was found to be replicating within infected beetles as evidenced by detection of minus-stranded RNA (Eyer et al. 2009b). Further studies should be conducted to verify whether small hive beetles actually cause outbreaks of SBV or other honey bee pathogens.

*Cryptic Reproduction.*

The presence of small hive beetles does not always correlate with observable damage (Spiewok and Neumann 2006a). Both adults and larvae can cause significant damage through their feeding habits within the colony; however, it has been reported that only the larvae pose a direct threat, and that European colonies can host hundreds or thousands of adult beetles without any apparent damage (Lundie 1940, Wenning 2001).

Adult beetles are capable of reproducing at very low levels within a hive (Spiewok and Neumann 2006a). Several developmental stages were found in the debris of the bottom board (Spiewok and Neumann 2006a). This suggests that larvae may be able to sustain themselves on the debris without access to pollen, honey, or brood (Spiewok and Neumann 2006a). Strong colonies that keep the bottom board debris-free are less likely to promote this sort of cryptic beetle reproduction (Spiewok and Neumann 2006a). Colonies that allow debris build-up on the bottom board are supplying beetles with refuge and food (Spiewok and Neumann 2006a). Bees should have access to all parts of the hive to facilitate removal of debris that could support low beetle populations (Spiewok and Neumann 2006a).

**Life History and Reproductive Biology.**

*Host Finding and Attractants.*

Upon emerging from the ground, adult beetles actively seek out honey bee colonies (Lundie 1940, Schmolke 1974). During their first few days as an adult, beetles are highly attracted to light and prone to flight (Lundie 1940). Schmolke (1974) found that adult beetles prefer to fly before or after dusk, with males flying earlier than females. However, Elzen et al. (2000b) found adults flying before sundown during the month of June in Florida. These Florida beetles were attracted to traps baited with honey, pollen, and live bees (Elzen et al. 2000b). Photoperiod may be one factor affecting flight, but it appears that other factors may be involved as well (Elzen et al. 2000b). While males seem to fly earlier than females, it may be that males are more responsive than females to certain cues (Elzen et al. 2000b). As adults age, they lose their attraction to light and become less active fliers, preferring to run instead of taking flight (Lundie 1940, Schmolke 1974). At 10 days old, adults showed no attraction to lights (Schmolke 1974). A study by Baxter et al. (1999) showed that adult beetles were not attracted to

incandescent, black, or insect light. The few that were attracted to these lights were probably recently-emerged adults.

Arbogast et al. (2009) observed that beetles were more attracted to baited flight traps that were located in full shade than to traps that were located in partial shade. A study in Louisiana found that beetles also preferred shaded colonies to sunlit colonies, even though the shaded and sunny colonies were relatively close to each other (de Guzman et al. 2010). Beetles may choose shaded colonies because they also occur near shaded soil (de Guzman et al. 2010). Shaded soil may be more suitable for pupation, as soil that is constantly exposed to the sun is more likely to result in larval and pupal desiccation (de Guzman et al. 2010).

It has been shown in flight-tunnel and olfactometric bioassays that volatiles from live worker bees, freshly collected pollen, unripe honey, and slumgum are all highly attractive to small hive beetles (Suazo et al. 2003). Beetles are capable of finding new host colonies by detecting these volatiles in the air (Suazo et al. 2003, Torto et al. 2007a). Beetles respond more strongly to volatiles from older workers (Suazo et al. 2003). As one might expect, adult beetles have a stronger response to volatiles produced by increasing numbers of honey bee workers (Suazo et al. 2003). Wind tunnel experiments showed that beetles were more attracted to comb with workers present than to worker-free combs (Torto et al. 2007a). In general, female beetles were more responsive than males, with the greatest disparity in tests with unripe honey (Suazo et al. 2003). Both sexes showed a stronger response to freshly-collected pollen than to commercially packaged pollen (Suazo et al. 2003). Even when honey was added to fresh and packaged pollen, beetles still showed greater responses towards fresh pollen (Suazo et al. 2003). Baited traps in the field showed that just hive products or just live worker bees were only weakly attractive to beetles (Elzen et al. 1999a). Adult beetles readily came to traps baited with pollen,

honey, and live worker bees (Elzen et al. 1999a, 2000b). It has been reported that adult beetles can detect stressed honey bee colonies from eight to 10 miles away (Wenning 2001), but no studies have been conducted to confirm this assertion.

Small hive beetles are also attracted to the chemicals in honey bee alarm pheromone (mainly isopentyl acetate, 2-heptanone, and methyl benzoate) (Torto et al. 2007a). Artificially stressed European colonies released 1,500- to 10,000-fold more alarm pheromone than unstressed colonies (Torto et al. 2007a). Male and female adult beetles were able to detect isopentyl acetate in amounts as low as 2 ng during coupled gas chromatographic-electroantennogram analyses (Torto et al. 2007a). Even guard bees and forager bees could not detect this amount of isopentyl acetate in the air (Torto et al. 2007a). However, isopentyl acetate alone is not a sufficient attractant and is only attractive in conjunction with other hive volatiles (Torto et al. 2007a). Adult beetles could also detect 11 other hive volatiles, while worker bees could only detect five (Torto et al. 2007a). The yeast *K. ohmeri* also produces alarm pheromone chemicals when grown on pollen, serving to attract beetles to the colony (Torto et al. 2007a, Benda et al. 2008). Hive volatiles, along with the volatiles produced by yeasts, serve as olfactory cues which allow beetles to home in on honey bee colonies (Torto et al. 2007a). The beetles' acute olfactory sensitivity apparently allows them to find even remote colonies.

*Mating.*

Very little is known about the mating behaviors of the small hive beetle. Females may potentially live for a year or more, but it is unknown whether they mate once or several times over the course of their lifetime (Ellis 2004). Several studies (Neumann et al. 2001b, Ellis et al. 2003f, Ellis 2005) have observed adult beetles mating in honey bee propolis prisons. It is likely that beetles mate within the colony in sheltered areas away from worker bees. It is not known if

beetles mate outside of the colony. They might mate shortly after eclosion before seeking out a host colony. Somerville (2003) noted that disturbing the environment elicited mating from beetles. Adult beetles confined to a jar began to mate after the jar was gently shaken (Somerville 2003). However, he also noted that moving bee colonies by truck caused many of the beetles to leave the hives (Somerville 2003). Whether mating occurred in truck-transported colonies is not known, but it seems that too much disturbance elicits a different response.

*Aggregation Pheromones.*

Thus far, no aggregation pheromones have been discovered for the small hive beetle (Torto et al. 2007a). However, other Nitidulids have been reported as having aggregation pheromones (Bartelt 1999). Because small hive beetles can detect other volatiles which may serve the same function, aggregation pheromones may not be necessary. The volatiles released from fermentation products, the yeast *K. ohmeri*, and honey bee alarm pheromone may serve as aggregation cues (Spiewok et al. 2007, Torto et al. 2007a).

*Oviposition.*

Several studies have reported different data on the time that it takes for adult beetles to reach sexual maturity. Lundie (1940) noted that it took nearly a week before female beetles would begin to lay eggs at an unreported temperature. Schmolke (1974) observed a shorter pre-oviposition period of two to four days at 30°C. Haque and Levot (2005) observed a period of three to six days at 29°C. Cuthbertson et al. (2008) noted that beetles began to lay eggs within three to four days at 30°C. Temperature may have an effect on the pre-oviposition period (Lundie 1940, Schmolke 1974, Haque and Levot 2005, Cuthbertson et al. 2008). However, although Lundie does not report his temperatures, he probably raised his specimens at room temperature. It is also important to note that a protein-rich diet is required for egg production

(Schmolke 1974, Ellis et al. 2002b). Each of the studies provided an adequate protein diet (usually in the form of a pollen-honey mixture). The variation in the pre-oviposition range may also be attributed to Schmolke's (1974) finding that some adults remain in the ground for several days before emerging. However, not all adults choose to remain in the soil, and some will emerge as soon as they eclose (Schmolke 1974). This pre-emergence period may not have been accounted for in some studies; rather, only time spent above the soil was recorded. Cuthbertson et al. (2008) also noted that some adults emerge, fed on provided honey, and reentered the soil to emerge again after one to two days had passed. If beetles are not watched closely, they may reenter the soil. Although they may be emerging from the soil for the second time, it may appear as if they had just eclosed.

Schmolke (1974) estimated that a single female beetle could lay more than 1,000 eggs over the course of three to four months. Somerville (2003) reported higher numbers, stating that a single female could lay 2,000 eggs in a year or more, laying up to 200 eggs per day. A study by Neumann et al. (2001a) reported that 300 laboratory beetles produced 3,800 larvae over the course of 21 days. Ellis et al. (2003a) observed that individual females laid more than 10 eggs per honey bee comb cell. In a study by Ellis and Delaplane (2008), adult females laid anywhere from 13 to 25 eggs per comb cell, on average. Ellis et al. (2003d) reported as many as 33 eggs per comb cell in some cases.

Somerville (2003) reported that eggs ceased to hatch at temperatures below 10°C. High relative humidity is also essential for the successful hatching of small hive beetle eggs, as they are vulnerable to desiccation (Schmolke 1974). Somerville (2003) reported that the ideal humidity level for eggs was 60% or higher. Schmolke discovered the fragility of these eggs while trying to raise beetle eggs on a piece of glass. Exposed to circulating air without high

humidity, the eggs readily desiccated. He later placed the eggs in a cardboard carton with a dish full of water along with some damp paper towels and found greater success with the increased humidity.

Ellis and Delaplane (2008) stated that the density of small hive beetles in a colony did not affect oviposition rate and number of eggs oviposited per cell by females. On the other hand, Somerville (2003) observed that adult females stopped ovipositing once there was a large population of larvae. It is not known how many larvae must be present before females will cease egg-laying. Perhaps Ellis and Delaplane (2008) did not reach that larval threshold. Other factors may also play a role in oviposition rate and density, such as beetle age, oviposition location, and pheromones (Ellis and Delaplane 2008). However, these factors have not yet been examined.

In a very early study by Elzen et al. (1999b), cooperators in Florida reported that small hive beetles were laying eggs on the backs of worker bees. It may be that the cooperators saw something on the bees that resembled eggs but were not actually eggs. This egg-laying behavior has not been witnessed by other researchers nor examined in any detail. Worker bees generally respond to beetles with aggression, so it is unlikely that beetles would climb on bees to lay their eggs.

Were it not for the honey bee workers present on the combs, female beetles would lay their eggs in and around pollen masses or in brood cells (Lundie 1940, Schmolke 1974). The worker bees tend to chase off any free-roaming beetles, forcing these beetles to oviposit in the cracks and crevices of the hive periphery (Lundie 1940, Schmolke 1974). Honey bees are very efficient at removing unprotected beetle eggs, serving as a selective pressure to lay eggs in areas where bees are unable to reach them (Schmolke 1974). The female small hive beetle is equipped with a long ovipositor and can lay eggs in very narrow and deep areas (Schmolke 1974). Should

a female beetle be allowed access to the comb, she can lay eggs on the bee brood. Ellis et al. (2004c) observed beetles laying eggs among the brood comb. Female beetles chewed a hole in a cell with their mandibles, positioned their abdomen above the hole, and then inserted their ovipositor to lay eggs (Ellis et al. 2004c). The entire egg-laying process can take as little as five seconds but usually takes additional time, depending on how many eggs the female decides to oviposit (Ellis et al. 2004c). Females can puncture the cell capping and lay eggs on the top of a honey bee pupa (Ellis and Delaplane 2008). Or if a brood cell has an empty adjacent cell, female beetles can puncture the wall of the brood cell and lay eggs through the side or bottom of the cell (Ellis and Delaplane 2008). Eggs can be laid directly on the bee pupa or on the cell wall surrounding the pupa (Ellis et al. 2003a). If a female oviposits from an empty adjacent cell, she reduces her chances of being molested by honey bee workers (Ellis and Delaplane 2008). Females rarely oviposited on bee brood from both sites (only 7% of sealed bee brood), but when oviposition occurred at both sites the number of beetles eggs per cell nearly doubled that of single-site ovipositing (Ellis and Delaplane 2008).

The temperature of the interior of the hive can range from 30-35°C, being roughly 34°C in the brood area and getting progressively cooler away from the center (Winston 1987). Because honey bee workers actively pursue beetles on the comb and thus keep most beetles from ovipositing in the center of the brood area, beetle eggs are most likely to be laid at the periphery of the brood area (Schmolke 1974). The majority of eggs hatch in about two days under normal hive temperatures (Lundie 1940, Somerville 2003, Haque and Levot 2005), although a smaller proportion of the eggs are capable of hatching in a single day (Ellis and Delaplane 2008). Lundie (1940) found that eggs that hatched after five days were still viable. When raised at room

temperature, three days were required for 98% hatching of eggs (de Guzman and Frake 2007). When raised at 30°C, 89% of eggs hatched within two days (Schmolke 1974).

*Larval Development.*

There appears to be some confusion about the number of instars present during small hive beetle development. Lundie (1940) and Schmolke (1974) made no mention of the number of instars. De Guzman and Frake (2007) reported three instars when beetles were raised at 34°C and also at room temperature (24-28°C). Haque and Levot (2005) reported four when raised at 29°C. Hood and Miller (2005) noted that "fifth instars of *A. tumida* larvae and pupae were observed in soil below two colonies." The number of instars may be influenced by nutrition and/or temperature. Regardless, larvae go through two phases: a feeding phase and a non-feeding phase (Lundie 1940, Schmolke 1974, Haque and Levot 2005, de Guzman and Frake 2007).

Here, we discuss larval development at 29°C as reported by Haque and Levot (2005). First and second instars mature in about one day (Haque and Levot 2005). Third and fourth instars take about two days to mature (Haque and Levot 2005). Larval feeding behavior changes during the fourth instar (Haque and Levot 2005). When the larvae are in the feeding phase, they are negatively phototaxic and prefer not to move far from their food source (Schmolke 1974). The feeding phase may last up to six days (Haque and Levot 2005). However, after the larvae have become fully-grown, they will enter a post-feeding, wandering stage and become positively phototaxic (Schmolke 1974). Wandering larvae may stay near their food source for up to 15 days, even though they ceased feeding around five to six days (Haque and Levot 2005). The wandering larvae will drop from the hive and pupate in the soil below (Lundie 1940, Schmolke 1974). Schmolke (1974) reported that larvae spent, on average, 13 days on the comb and three days in the soil at 30°C.

Wandering larvae will go through great lengths to find a suitable pupation site. Schmolke (1974) witnessed larvae wandering up to 30 meters before finding a place to burrow. Somerville (2003) estimated that wandering larvae could travel hundreds of meters under ideal conditions. Ellis et al. (2004) noted that even when deprived of a place to pupate, wandering larvae could survive for more than a month as long as they were kept moist (i.e. high humidity). In Africa, Schmolke (1974) noted that even when hives were placed on large flat boulders or other unsuitable pupation substrates, wandering larvae could still successfully travel several meters in moist or cool conditions. Once in the soil, larvae construct smooth-walled cells and enter the pre-pupal stage (Lundie 1940). If soil conditions change unfavorably before the larvae reach the pre-pupal stage, the larvae will relocate to a more suitable location (Schmolke 1974). In a typical apiary environment, the majority of larvae, pupae, and eclosing adults are found within 30 centimeters of the hive entrance (Pettis and Shimanuki 2000).

*Pupal Development.*

*Depth of Burrowing.*

Wandering larvae take about three minutes to burrow once they reach suitable soil (Mürrle and Neumann 2004). In the United States, the vast majority of pupae can be found within the top 10 centimeters of soil (Pettis and Shimanuki 2000). Mürrle and Neumann (2004) found that lab-raised larvae would burrow up to 15 centimeters in depth. Pettis and Shimanuki (2000) recorded larvae as deep as 20 centimeters. De Guzman et al. (2009) observed that the depth of burrowing did not appear to be affected by soil type. However, Torto et al. (2010) noted that soil moisture did have an effect on the depth of burrowing. Small hive beetle larvae in Kenya would burrow deeper during dry conditions (Torto et al. 2010). During wet conditions in

Kenya, larvae burrowed zero to 15 centimeters into the soil (Torto et al. 2010). Under dry conditions, larvae burrowed 11 and 20 centimeters (Torto et al. 2010).

*Temperature and Pupation Length.*

Lundie (1940) reported that most pupae remained in the soil for three to four weeks at an unreported temperature. At 29°C, most adults emerged after 16 days in the soil, but a small proportion emerged in as little as 13 days to as late as 25 days (Haque and Levot 2005). At 30°C, Schmolke (1974) found that adult beetles emerged after spending 15 to 17 days in the soil. At 20-30°C, Cuthbertson et al. (2008) found that beetles emerged as early as 18 days and as late as 84 days. Females develop faster than males at room temperature (de Guzman and Frake 2007). Regardless of temperature, small hive beetles spend most of their developmental time in the soil (>75%) (de Guzman and Frake 2007).

*Effect of Soil Type.*

There is some debate about whether soil type greatly affects pupation; however, it appears that successful pupation can occur among all soil types (Lundie 1940, Schmolke 1974, Somerville 2003, Ellis et al. 2004a, de Guzman et al. 2009). Soil type did affect the length of time that pupae spent in the soil (Ellis et al. 2004a). Schmolke (1974) found greater pupation success with moist sand and moist loam but less with moist clay loam. De Guzman et al. (2009) observed greater beetle populations in an apiary with silty clay and silty clay loam than in an apiary with predominantly loamy soil. Pettis and Shimanuki (2000) reported that most larvae in Florida were found in moist sandy soils. It is also interesting to note that small hive beetles cause more damage in Florida than other state in the U.S. (Neumann and Elzen 2004); but temperature, high humidity, and rainfall may also play a role in their success in Florida (Somerville 2003). In

contrast, Ellis et al. (2004a) found that adult emergence occurred equally well in loamy sand, silty clay, sandy loam, and clay loam.

*Effect of Soil Moisture.*

The importance of soil moisture was first noted by Lundie (1940). He found that pupal mortality increased when there was free air flow through the soil chambers (Lundie 1940). Schmolke (1974) noted that there was no adult emergence from dry soils but high adult emergence from moist soils. Somerville (2003) reported that wandering larvae and pupae required a soil moisture level between 5% and 25%. Wandering larvae placed on dry, tilled soils died within nine days (Ellis et al. 2004a). These larvae may have succumbed to dehydration or asphyxiation from the dusty conditions (Ellis et al. 2004a).

*Effect of Soil Compaction.*

Soil compaction has mixed effects on pupation success (Ellis et al. 2004a). Wandering larvae tend to burrow within a minute of being placed on top of suitable, loosely packed soil (N. Wright, personal observation). Larvae placed on moist packed soils take significantly longer to begin burrowing, but those that burrowed emerged successfully as adults (Ellis et al. 2004a). Compact soils may make burrowing more difficult and require more energy than tilled or loosely packed soils (Ellis et al. 2004a). Soil conditions may be one reason why small hive beetles have become a problem in invaded areas (Ellis et al. 2004a). The arid and semiarid soils of Africa may minimize adult emergence rates, resulting in lower populations (Ellis et al. 2004a). In Florida, for example, the moist sandy soils provide ideal pupation conditions which may allow for huge populations to develop.

*Effect of Temperature on Overall Development and Survival.*

It has been shown that temperature has marked effects on developmental times (Schmolke 1974, de Guzman and Frake 2007). Most of the confusion about small hive beetle development stems from the wide range of temperatures used in many studies. Studies that concentrate on raising beetles at temperatures that most closely mimic the hive environment (30-35°C) are useful for determining developmental rates in managed bee colonies; however, abandoned hives will have great temperature fluctuations, as there are no workers present to regulate and maintain a constant hive temperature. Also, once wandering larvae leave the colony, they are susceptible to the varying temperatures of their soil environment. Developmental variation due to temperature is most noticeable when viewing the entire small hive beetle life cycle from egg to adult.

Lundie (1940) was the first to study small hive beetle development in detail. He reported a range of 31 to 80 days from egg to adult but at an unspecified temperature (Lundie 1940). Schmolke (1974) reported an average of 32 days at 30°C, a temperature which reflects the periphery of the hive. Haque and Levot (2005) reported 24 to 46 days at 29°C. De Guzman and Frake (2007) found that it took an average of 23 days to develop at 34°C, a temperature that would accurately reflect the center of the brood comb. Mürrle and Neumann (2004) found that development took 40 to 43 days at 18-25°C (room temperature). Neumann et al. (2001a) reported about 49 days at 17-24°C (room temperature).

Somerville (2003) reported that larval survival rates decreased as temperature decreased. At 30°C, nearly all larvae reached maturity (Somerville 2003). At 20°C, more than half of the larvae died (Somerville 2003). At 10°C, no larvae survived to maturity (Somerville 2003).

*Overwintering.*

African honey bees do not need to overwinter like their European counterparts (Hepburn and Radloff 1998). It would be expected that small hive beetles would be unable to survive in colder regions because they are tropical in origin and not adapted for overwintering (Pettis and Shimanuki 2000). However, small hive beetles have been found successfully overwintering in European honey bee clusters (Pettis and Shimanuki 2000, Hood 2004, Neumann and Elzen 2004). Apparently, small hive beetles are able to reproduce year-round in Florida but must cease reproduction and overwinter in more northern states such as Georgia and South Carolina (Pettis and Shimanuki 2000). Adult beetles are the only stage that can overwinter; larvae are not present in hives and pupae are absent from the soil during winter months (Pettis and Shimanuki 2000). Pettis and Shimanuki (2000) found over 300 beetles overwintering in a single hive cluster. Ellis (2004) found that only a small number of overwintering beetles were present outside of the cluster. Of these, more than 75% were within 5 cm of the cluster perimeter (Ellis 2004). Beetles within the cluster were found hiding in cells, with some cells containing more than five beetles (Ellis 2004). Although adult beetles are able to obtain warmth from the honey bee cluster, a recent study conducted in Maryland showed that only a small percentage of adult beetles successfully overwinter (Schäfer et al. 2010a). Their effect on honey bee winter losses is insignificant (Schäfer et al. 2010a). Data from a study conducted in Louisiana (de Guzman et al. 2010) suggest that majority of adult beetles do not overwinter in the cluster. It was observed that most beetles died during the winter or left their host colonies (de Guzman et al. 2010). Adult beetles cease movement at temperatures below 21°C (Somerville 2003). At some point, adults will die to freezing, but this temperature has not yet been determined scientifically (Somerville 2003).

*Population Dynamics.*

In South Africa, Lundie (1940) found that small hive beetles produced five generations a year. He also noted that no reproduction occurred during the colder months of April to August (Lundie 1940). Schmolke (1974) reported that adults could be found throughout the year in Rhodesia. Torto et al. (2010) also reported finding adult beetles year-round in Kenya at low numbers. In Kenya, beetle numbers peaked during the rainy season (Torto et al. 2010). Eighty percent of adult beetles were captured during this time, while the remaining 20% were captured during the drier parts of the year (Torto et al. 2010). Larval survival was significantly higher during wet conditions than dry conditions (Torto et al. 2010).

Somerville (2003) reported that small hive beetles could go through six generations in a year under moderate U.S. and South American climatic conditions. In Louisiana, beetle populations peaked during the autumn months of September and November (de Guzman et al. 2010). High summer temperatures may shorten beetle developmental time (de Guzman et al. 2010). In areas of the U.S. that have mild winters, beetles are expected to complete more generations per year (de Guzman et al. 2010). The high beetle populations during the autumn may also be attributed to honey bee colonies that died during the summer (de Guzman et al. 2010). Regardless of how these colonies failed, beetles can infest any dead, unprotected colonies and produce vast quantities of offspring (de Guzman et al. 2010). A later study by de Guzman et al. (2011) reported capturing most adult beetles during April and May with very few trapped during the winter and fall. In La Crosse, Florida, most beetles were caught in the spring and summer months with peak captures in May and June (Arbogast et al. 2009b). In Louisiana, it was noted that the worst larval damage occurred during the summer from June to August (de Guzman et al. 2010).

**Diet.**

*Natural Diet and Longevity.*

The small hive beetle is a scavenger of honey bee colonies (Lundie 1940, Schmolke 1974). Both the adults and larvae feed on pollen and honey (Lundie 1940, Schmolke 1974); however, they will preferentially consume bee brood and eggs, even in the presence of bee products (Schmolke 1974, Elzen et al. 1999a, Neumann et al. 2001a). According to Ellis et al. (2002b), honey-fed adults can live for an average maximum of 167 days (roughly five and a half months). Lundie (1940) reported similar results, stating that adults could survive for more than five months when fed only honey. Somerville (2003) estimated that adult beetles could live for more than a year in the wild. He also noted that some laboratory-reared specimens survived for as long as 16 months, although no mention is made of the nutrition these beetles were provided (Somerville 2003). Although adults can sustain themselves on honey alone, larvae require a source of protein in order to develop successfully (Lundie 1940). If the larvae are protein-starved, they may resort to cannibalism to obtain their needed protein (Lundie 1940).

Adult beetles can survive between 10 to 14 days without any food (Neumann et al. 2001b, Ellis et al. 2002b). Adult beetles were able to survive for upwards of 50 days on brood comb alone (Ellis et al. 2002b). However, it is unlikely that these beetles survived by consuming the wax. Rather, they may have fed on old pupal cocoons or other debris in the brood comb until they ran out of food (Ellis et al. 2002b).

Female beetles are unable to reproduce without a source of protein in their diet (Ellis et al. 2002b). Schmolke (1974) noted that beetles performed well on diets containing at least pollen and water. No reproduction occurred when adults were fed just honey, sucrose sugar crystals, water, or empty brood comb (Ellis et al. 2002b, Haque and Levot 2005, Buchholz et al. 2008).

High reproductive potential was seen in adults fed pollen-rich diets (Ellis et al. 2002b). Arbogast et al. (2009b) found that adults which fed on pollen dough inoculated with the yeast *K. ohmeri* were reproductively superior to adults fed pollen dough without the yeast. Thus, it is thought that *K. ohmeri* may provide additional nutrients for the beetles (Arbogast et al. 2009b). Bee brood is also high in protein and is preferred over many other bee products (Buchholz et al. 2008). However, the adult per larvae ratio did not differ significantly from pollen, pollen-honey, and brood diets, so it appears that these diets are equally appropriate for larval and pupal fitness (Ellis et al. 2002b).

*Alternative Diets.*

*Fruits and Artificial Diets in the Laboratory.*

Studies have shown that small hive beetles are able to survive and reproduce successfully on food sources other than honey bee products (Ellis et al. 2002b, Buchholz et al. 2008). Schmolke observed this firsthand in 1974 when two escapee adult beetles were found feeding on grapes in a fruit bowl. Ellis et al. (2002b) noted that adults could survive for more than two months on diets of fruit. In the laboratory environment, these beetles have been found to reproduce on fresh and rotten Kei apples, bananas, grapes, mangos, avocadoes, cantaloupes, oranges, grapefruits, and artificial *Manduca* diet (Eischen et al. 1999a, Ellis et al. 2002b, Keller 2002, Buchholz et al. 2008, Arbogast et al. 2009b).

Compared to a pollen-honey diet, most fruits resulted in higher adult mortality and/or reduced reproductive success (Buchholz et al. 2008). In a study done by Buchholz et al. (2008), about 17% of adults died on mangos and bananas and 50% died on grapes. Fungal growth and fruit decay may have influenced adult mortality in these fruit studies (Buchholz et al. 2008). Reproduction on fruits and *Manduca* diet was shown to be highly reduced compared to a pollen-

honey diet (Ellis et al. 2002b, Keller 2002, Buchholz et al. 2008), except in the case of inoculated oranges (Arbogast et al. 2009b). Arbogast et al. (2009b) found that oranges inoculated with the yeast *K. ohmeri* was an excellent diet that yielded similar reproductive results to inoculated pollen dough. Oranges that were not inoculated resulted in faster development to adulthood but also resulted in greater larval mortality (two-fold more) when compared to inoculated pollen dough (Arbogast et al. 2010).

The lower overall protein content of fruit may affect reproductive capacity (Roulston et al. 2000, Buchholz et al. 2008). Despite this, adult beetles still chose to oviposit on fruits even when supplied with bee products (Buchholz et al. 2008). When provided with four oviposition substrates, adults chose to oviposit mainly on pollen and bananas over both strawberries and honey (Buchholz et al. 2008). However, when bee brood was present, adults oviposited preferentially on brood (Buchholz et al. 2008). The high moisture content of bananas may prevent desiccation of eggs and larvae (Buchholz et al. 2008). Compared to other fruits, bananas are relatively high in protein and may be suitable for developing larvae (Buchholz et al. 2008). Bananas also contain isopentyl acetate, a component found in honey bee alarm pheromone (Torto et al. 2007a, Buchholz et al. 2008). However, Torto et al. (2007) noted that isopentyl acetate was only attractive in the presence of other hive volatiles; isopentyl acetate alone did not elicit a strong response from adults. Their preference for bananas over other fruits is likely based on several factors and not just the presence of isopentyl acetate.

*Fruits in the Field.*

Laboratory studies might suggest that small hive beetles can survive on wild fruits; however, field studies suggest that these beetles seldom make use of fruits in the field (Eischen et al. 1999a, Buchholz et al. 2008). A study by Buchholz et al. (2008) found that ant-protected

fruit buckets attracted mainly other sap beetles (mostly *Carpophilus*) and fruit flies. Small hive beetles were rarely observed in the buckets (Buchholz et al. 2008). Larvae were only found in fruit buckets when adults were under confinement (Buchholz et al. 2008). Adults confined to these buckets showed very little reproductive success (Buchholz et al. 2008). It is possible that these other sap beetles, fruit flies, and foraging ants may outcompete small hive beetles on fruit (Buchholz et al. 2008). Free-roaming small hive beetles were not observed ovipositing on fruit buckets near apiaries (Buchholz et al. 2008). Perhaps in the absence of colonies, fruits may provide an alternative food source for small hive beetles in the wild (Buchholz et al. 2008). However, it is not known whether an individual fruit will persist long enough for a beetle to lay eggs, for these eggs to hatch, and for larvae to complete the feeding phase, especially in the presence of other, more specialized fruit feeders (Eischen et al. 1999a). Because these beetles have never been reported as a fruit pest in South Africa (M. F. Johannsmeier, unpublished data in Neumann and Elzen 2004), these food sources are unlikely to result in high populations (Buchholz et al. 2008). Eischen et al. (1999a) also noted that citrus trees near apiaries did not have beetle infestations. However, when cut fruit was placed amidst a dead apiary of 90+ colonies in Florida, beetles arrived (Eischen et al. 1999a). Nearly 200 beetles flocked to cut cantaloupe and 100 more came to cut pineapple (Eischen et al. 1999a). Even more beetles were found among a whole cantaloupe; some 500 beetles were found inside the fruit after having chewed their way through the tough rind (Eischen et al. 1999a). In the absence of colonies, these beetles become opportunistic feeders (Eischen et al. 1999a, Ellis et al. 2002b, Buchholz et al. 2008). Because beetles can, at a minimum, sustain themselves on fruits, it is possible that fruit transporters could spread small hive beetle to other un-infested areas (Ellis et al. 2002b). Arbogast et al. (2009b) believe that small hive beetles are "ecological generalists able to

maintain adequate levels of reproduction in marginal environments but able to reach high levels in favorable, resource rich environments, such as honey bee colonies."

*Flowers.*

It is unlikely that small hive beetles are able to sustain themselves on flowers alone (Buchholz et al. 2008). In field trials with flowers, small hive beetles confined to two blooming species of Asteraceae (*Rudbeckia hirta* L var. "Goldilocks" and *Coreopsis verticillata* L var. "Zagreb") died within 14 days (Buchholz et al. 2008). Although the beetles had access to water and shelter, it appeared that they were unable to take advantage of the floral nectar and pollen (Buchholz et al. 2008). No reproduction occurred on either flowering plant (Buchholz et al. 2008). In addition, surveys of five flowering plants (*Rosa canina*, *Romneya coulteri*, *Chrysanthemum leucanthemum*, *Gardenia augusta*, and *Rosa* sp.) on the campus of the University of Western Sydney, Australia, failed to find any adult small hive beetles (Buchholz et al. 2008). A related species, *Aethina concolor*, was collected 13 times during the surveys (Buchholz et al 2008). This endemic species was observed on *R. coulteri* and *G. augusta* (Buchholz et al. 2008). While flowers probably do not constitute a suitable alternative food source for the small hive beetle, other families and species of flowering plants should also be tested to confirm this inference. It may also be worthwhile to examine beetle activity on flowering plants native to sub-Saharan Africa.

**Interaction Between Beetles and Honey Bees.**

Schmolke (1974) was the first to observe the interactions between small hive beetles and African honey bees in a glass-walled observation hive. While African honey bees differ from their European counterparts, they share in the same set of behavioral defense strategies. These defensive behaviors are not specific to any particular hive pest; rather, these behaviors are part of

a general reaction to any small nest invader (Ellis et al. 2003e). Honey bees have the following defensive strategies which they use against small hive beetles: aggression and patrolling (Lundie 1940, Schmolke 1974, Elzen et al. 2001), removal of beetle eggs and infested brood cell contents (Ellis et al. 2003d, Neumann and Härtel 2004, Spiewok and Neumann 2006), removal of beetle larvae (Lundie 1940, Schmolke 1974, Spiewok and Neumann 2006), social encapsulation (Neumann et al. 2001b; Ellis et al. 2003e, 2003f), and absconding (Hepburn and Radloff 1998, Hood 2000). Some of the differences in the susceptibility between European honey bees and African honey bees may be explained by the differences in these defensive behaviors (Neumann and Elzen 2004).

*Aggression and Patrolling.*

*Honey Bee Behavior.*

Aggression is the most obvious reaction to free-roaming adult beetles in the hive (Lundie 1940, Schmolke 1974). Upon introducing adult beetles into a beetle-free colony, Schmolke (1974) noticed that African worker bees would chase nearby beetles and attempt to catch them with their mandibles and feet. The chase was usually short-lived; workers would only pursue for a few centimeters before disengaging (Schmolke 1974). Should a free-roaming adult be caught successfully, the consequences are fatal (Schmolke 1974). Schmolke observed several workers cooperatively pulling off the extremities of an adult beetle (Schmolke 1974). The pieces were then removed from the hive (Schmolke 1974, Neumann et al. 2001b). However, the capture of an adult beetle seldom occurs. Workers also attempt to sting adult beetles, but the tough and sleek exterior of the beetle prevents most stings from penetrating (Lundie 1940, Schmolke 1974).

African honey bee workers are, at times, relentless in their pursuit of beetles. If a beetle successfully infiltrates the comb area and hides in a cell, alerted African bees will remove nearby honey, pollen, and brood to get to the beetles (Schmolke 1974, Neumann and Elzen 2004).

Several studies have shown that honey bee workers actively patrol to keep small hive beetles away from brood comb (Lundie 1940, Schmolke 1974, Spiewok et al. 2007). Adult beetles were never found resting or feeding in any area inhabited heavily by African worker bees (Schmolke 1974). Strong, well-populated colonies rarely ever had adult beetles on the comb (Swart et al. 2001). Weakened, less populous colonies were less able to defend against beetles and thus had more beetles on the comb (Lundie 1952). Patrolling behavior mainly occurs in the brood area (Schmolke 1974, Solbrig 2001) and diminishes towards the outer frames and honey supers (Neumann and Elzen 2004). Worker bee density may contribute to small hive beetle resistance, because more workers are able to harass beetles attempting to gain access to the combs (Neumann and Elzen 2004).

Cape honey bees are significantly more aggressive toward beetles than European honey bees (Elzen et al. 2001). Cape honey bees responded aggressively to free-roaming beetles 32.8% of the time, ignored beetles 30.6% of the time, and initiated antennal contact (but moved away from the beetle) 36.6% of the time (Elzen et al. 2001). European honey bees, on the other hand, responded aggressively only 1.4% of the time, ignored beetles 86.8% of the time, and initiated contact 11.7% of the time (Elzen et al. 2001). While Cape honey bees had their responses split nearly equally across all three responses, European bees preferred to ignore beetles most of the time (Elzen et al. 2001). This European honey bee docility may play a role in their vulnerability to small hive beetles (Elzen et al. 2001).

*Beetle Behavior.*

Adult beetles respond to worker bee aggression in four ways: defensive posture, running, dropping, and hiding (Schmolke 1974, Neumann and Elzen 2004). Beetles can squat down and hide their head, antennae, and legs from the grasping mouthparts of worker bees (Lundie 1940, Schmolke 1974, Neumann et al. 2001b). Beetles can also run from alerted bees, but never running farther than necessary unless the bees are intent on pursuing (Schmolke 1974, Neumann et al. 2001b). Beetles can also drop from the comb to lose their pursuer (Schmolke 1974). Typically, most beetles stay hidden to reduce their interactions with worker bees (Schmolke 1974). Beetles gather in small cracks (usually 2-4 mm gaps), take shelter in empty cells, or hide on the bottom board (Lundie 1940, Schmolke 1974, Neumann et al. 2001b).

It is interesting to note that beetles are only able to oviposit in African honey bee brood comb when there are no workers present (Ellis 2004). This is not the case in European colonies, as beetles are able to lay eggs in the brood comb despite the presence of worker bees (Ellis 2004).

*Removal of Eggs and Infested Brood Cell Contents.*

*Honey Bee Behavior.*

Because small hive beetle eggs can hatch within one to two days (Lundie 1940, Somerville 2003, Haque and Levot 2005, Ellis and Delaplane 2008), it is important that honey bees remove them quickly to prevent damage caused by hatching larvae (Neumann and Härtel 2004). The exact mechanism by which worker bees detect beetle eggs and larvae within brood cells is unknown, but there are many proposed explanations (Ellis et al. 2003d). Honey bees may be able to detect beetle eggs through olfactory cues (de Guzman et al. 2008), but there may exist a minimum number of eggs per cell that elicits removal of the cell contents (Ellis et al. 2003d, 2004c). Other olfactory cues, such as volatiles produced by bee brood from larval feeding (de

Guzman et al. 2008), oviposition chemicals released by female beetles (Ellis et al. 2004c), and frass volatiles from young larval beetles (Weiss 2006, de Guzman et al. 2008) may assist worker bees in detecting infested cells. However, it appears that workers do not cue into punctures on the cell wall or cell capping; workers did not accidentally remove brood from artificially-punctured cells or cells punctured by beetles but not oviposited in (Ellis et al. 2003d, 2004c).

Honey bees are very effective at removing unprotected eggs but have more difficulty in removing protected eggs, such as those hidden in small cracks and gaps (Neumann and Härtel 2004). African honey bees removed most unprotected eggs within one hour (72 ± 26%) and all unprotected eggs within 24 hours (Neumann and Härtel 2004). However, after 24 hours, a large percentage of protected eggs remained (66 ± 12%) (Neumann and Härtel 2004). It is much more likely that protected eggs will hatch, so it is essential that honey bees also be efficient in removing larvae to resist infestation by small hive beetles (Lundie 1940, Schmolke 1974, Neumann and Härtel 2004). Colony phenotype (size, amount of open and sealed brood, pollen and honey stores) was not correlated with the egg removal rate in African honey bee colonies (Neumann and Härtel 2004).

Honey bees can detect the presence of beetle eggs in capped brood cells (Ellis and Delaplane 2008). Within 24 hours, European honey bees removed the contents of 77% of infested cells (Ellis and Delaplane 2008). The removal of cell contents after 24 hours is less desirable, as the eggs may hatch within that time and have the opportunity to relocate or hide from workers (Ellis and Delaplane 2008). Colonies show significant variation in their removal rates (10.6 to 77.2%), but these rates were not affected by colony phenotype (number of adult bees and $cm^2$ of brood) (Ellis and Delaplane 2008). The results of these two studies (Neumann

and Härtel 2004, Ellis and Delaplane 2008) suggest that egg removal is not dependent on colony strength (Ellis and Delaplane 2008).

Russian honey bees and commercial European honey bee stock (mostly *A. m. ligustica*) had very similar removal rates for egg- and larvae-infested brood cells (de Guzman et al. 2008). Within six hours, both honey bee stocks removed the contents of 40% to 50% of infested cells (de Guzman et al. 2008). After 20 hours, European honey bees removed 81.87 ± 3.41% of infested brood cell contents (de Guzman et al. 2008). In the same time frame, Russian honey bees removed 82.0 ± 3.73% of infested brood cell contents (de Guzman et al. 2008). Complete removal of all infested cell contents was never observed (de Guzman et al. 2008). Both stocks exhibited an early and quick response to infested brood cells which reduces the amount of damage that can be caused hatching beetle larvae (de Guzman et al. 2008). Neither stock was superior over the other in their ability to detect, uncap, and remove infested brood (de Guzman et al. 2008).

Cape and European honey bees were also found to have similar removal rates for infested cell contents (Ellis et al. 2004c). Both subspecies preferentially removed perforated cells containing beetle eggs (Ellis et al. 2004c). Small hive beetles laid significantly more eggs per cell in Cape honey bee colonies (14.5 ± 1.4) than in European colonies (7.3 ± 0.4) (Ellis et al. 2004c). Because European honey bees are not the native host for small hive beetles, reduced egg-laying may be attributed to a lack of some chemical oviposition stimulant that is present in African honey bee colonies (Ellis et al. 2004c).

The data show that African, Cape, European, and Russian honey bees can all effectively remove infested brood (Neumann and Härtel 2004, Ellis et al. 2004c, de Guzman et al. 2008, Ellis and Delaplane 2008). While European colonies are more susceptible to beetle depredation

than both African and Cape colonies, the weakness of European honey bees does not appear to be caused by a lack of hygienic behavior (Ellis et al. 2004c). Other factors are likely to play a larger role in the European honey bees' vulnerability to small hive beetles.

*Removal of Larvae.*

*Honey Bee Behavior.*

The larvae of the small hive beetle are much less active than the adults. Workers are able to remove beetle larvae so long as the larvae are accessible to the workers (Lundie 1940, Schmolke 1974). The workers will grasp a larva in their mandibles, leave the hive, and fly several meters away to jettison the larva (Lundie 1940, Schmolke 1974). If the larva was not in the wandering stage, it will most likely starve, desiccate, or be eaten by predators. Potentially, a wandering larva would still be able to survive as long as it could find a suitable place to pupate.

In one case documented by Schmolke (1974), two bees seized hold of the same larva. They pulled at it until it split into two pieces, and then each bee flew off with their piece and jettisoned it far from the hive. Schmolke (1974) artificially introduced larvae into an uninfested hive and found that the bees removed half of the larvae within 90 minutes. Within 24 hours, all larvae had been removed (Schmolke 1974).

*Social Encapsulation.*

*Honey Bee Behavior.*

Honey bees are unable to reliably kill or eject adult beetles from the hive. In response to this, they have evolved a complex mechanism to limit adult beetle movement and reproduction. African, Cape, and European honey bees all engage in social encapsulation, a cooperative effort of imprisoning adult beetles with propolis within the hive (Neumann et al. 2001b, Ellis et al. 2003e).

Most beetles take shelter in the periphery of the hive. Bees that detect these beetles will work to encapsulate them in propolis, tree resin that is used to seal up small spaces and waterproof the hive (Neumann et al. 2001b, Ellis 2002, Ellis et al. 2003e). While some bees work to add propolis walls, other bees keep the beetles from escaping (Neumann et al. 2001b). Propolis prisons can vary greatly in their capacity, holding as few as one beetle to as many as 200 (Neumann et al. 2001b). Strangely, bees never completely walled off imprisoned beetles and always left a small gap in the propolis wall (Ellis 2002). Guard bees station themselves at the gap and attempt to keep adult beetles from escaping (Ellis 2002, Ellis et al. 2003b). At night, beetles are considerably more active (Ellis et al. 2003e) and take advantage of the reduced activity of the bees (Neumann et al. 2001b). It is during this time that the beetles attempt to escape from their prisons, and occasionally they succeed (Neumann et al. 2001b). Honey bee workers appear to respond to the increased beetle activity by stationing more guard bees at night (Ellis et al. 2003e). The increase in guard bees at night may also be related to the increase in foraging bees that have returned to the hive (Ellis et al. 2003b). Workers of the same age cohort are capable of multitasking, and they switch roles depending on the current needs of the colony (Winston 1992). It is possible that foraging bees may switch to guarding when they return home in the evening, although most will rest so they can forage the next day (Moritz and Southwick 1992). Heavily infested colonies experience a decline in foraging activity, supporting the argument that foragers and guards interchange roles as necessary (Ellis et al. 2003c). While social encapsulation can prevent beetle reproduction, it requires the attention of many workers to accomplish this goal (Neumann et al. 2001b).

For Cape honey bees, there were 2 ± 1.27 guard bees aggressively preventing beetle escape for each worker laying down propolis walls (Neumann et al. 2001b). These guard bees

kept beetles herded for up to 57 days while waiting for other workers to add propolis (Neumann et al. 2001b). Out of 40 infested Cape honey bee colonies, only eight had any free-roaming beetles (n = 32) (Neumann et al. 2001b). Out of these eight colonies, only two engaged in social encapsulation, but these two colonies also had the highest number of beetles (Neumann et al. 2001b). This suggests that honey bees only engage in social encapsulation when beetle numbers reach a certain threshold (Neumann et al. 2001b). No larvae were found in any of the 40 infested Cape honey bee colonies (Neumann et al. 2001b). Even though beetle reproduction was successfully prevented, Cape honey bees still absconded (Neumann et al. 2001b). For African and Cape bees, social encapsulation may provide workers bees time to prepare for absconding without being overrun by beetles and their larvae (Neumann et al. 2001b). It appears that even though social encapsulation is successful, the time and energy required to contain the beetles is very taxing on the colony, and absconding may be a more suitable option than coping with the infestation (Neumann et al. 2001b).

European colonies had fewer prisons and fewer beetles per prison (Ellis et al. 2003e) than Cape colonies (Neumann et al. 2001b). This may indicate that European honey bees are inferior to Cape honey bees when it comes to constructing and maintaining propolis prisons (Ellis et al. 2003e). Hepburn and Radloff (1998) also noted that European honey bees used less propolis than African honey bees. Even still, European colonies of 8,000 workers were able to prevent 93% of beetles from accessing the combs (Ellis et al. 2003e). However, only 25 beetles were introduced into each colony in that particular study (Ellis et al. 2003e). The results show that European colonies may be effective at resisting small hive beetle infestation, although this may only be the case at low population levels (Ellis et al. 2003e). Although Neumann et al. (2001b) reported that beetles are more successful at escaping from their prisons at night, Ellis et al. (2003b) reported

that, at least in European colonies, beetles did not escape more often during the morning or evening. European honey bee guards were not more aggressive at night, even though the number of guard bees per prison and per imprisoned beetle did increase (Ellis et al. 2003e). European workers were also noted to engage in "prison wall-working," which involved workers touching the propolis walls with their front legs and also biting at it with their mandibles (Ellis et al. 2003e). The function of this behavior is not fully understood, but the bees may be gauging the quality or durability of the walls (Ellis et al. 2003e). Workers performed prison wall-working less at night, perhaps focusing their attention on guarding more active beetles (Ellis et al. 2003e).

Small hive populations that gradually build up may be more manageable for honey bees than rapid ones (Ellis 2002). Beekeepers of infested European colonies often experienced worse beetle problems after opening their hives (Ellis 2002). This may disrupt beetle prisons, allowing adult beetles to escape into the hive and begin reproducing (Ellis 2002). Although European honey bees are able to socially encapsulate beetles like their African counterparts, it is unknown whether their efficiency decreases at high beetle populations (Ellis et al. 2003e). Since European honey bees appear to exhibit variation in social encapsulation, it may be possible to select and breed for traits that enhance their ability to encapsulate beetles (Ellis et al. 2003e).

*Beetle Behavior.*

While imprisoned, small hive beetles are able to survive longer than two months (Neumann et al. 2001b). Because adult beetles can only survive for 10 to 14 days without any food, the beetles must have been acquiring food from some source (Neumann et al. 2001b, Ellis et al. 2002b). Mating and cannibalism also occur in the prisons but reproduction does not (Neumann et al. 2001b). Cannibalism may extend the life of some beetles in prison, especially in prisons with high inmate populations (Neumann et al. 2001b). In addition to cannibalism, these

beetles also rely on behavioral mimicry in order to survive in these prisons (Neumann et al. 2001b; Ellis et al. 2002c, 2003e). Adult beetles approach guard bees, extend their heads, and attempt to make antennal contact (Ellis et al. 2002c). While most soliciting beetles are met with aggression and are forced to withdraw, some successful beetles are rewarded with honey (Ellis et al. 2002c). Duped guard bees will regurgitate honey to a beetle, believing it to be a hungry worker trying to engage in trophallaxis (Ellis et al. 2002c). It usually takes between five and eight attempts to successfully solicit food (Ellis 2002, Ellis et al. 2002c). In both Cape and European colonies, beetles acquired more food from trophallaxis during the evening (Solbrig 2001, Ellis et al. 2003e). This is not necessarily because beetles are more convincing mimics at night; rather, more guard bees are stationed at each prison at night which led to increased trophallactic contact (Ellis et al. 2003e). Chemical mimicry may also be involved in inducing trophallaxis, but no studies have been conducted to confirm this (Ellis et al. 2002c).

**Absconding.**

*Honey Bee Behavior.*

In their native range, small hive beetles attack weakened/diseased colonies and scavenge abandoned colonies after an absconding event (Ellis et al. 2003b). African honey bee races are known to readily abscond (a form of non-reproductive swarming) and will leave behind brood and food stores (Hepburn and Radloff 1998). While several factors can lead to absconding, infestation by parasites is one such factor (Hepburn and Radloff 1998). Slight nest destruction and fermentation of some of the honey stores can induce absconding as well (Neumann and Elzen 2004). In contrast, European honey bees rarely abscond, even when faced with severe infestations and nest predation (Winston 1992). Because the African races are able to tolerate large numbers of beetles with only minor damage, one would imagine that frequent absconding

would not be necessary (Neumann and Elzen 2004). It may be that in time, nest efficiency is greatly reduced as a high portion of bees are relegated to guard duty to control the beetles (Hepburn and Radloff 1998).

Both European and Cape honey bees were found to abscond when subjected to high levels of small hive beetles (1,400 beetles total, 100 beetles a day for 14 days) (Ellis et al. 2003b). Only 10% of European control colonies absconded while 44% of Cape control colonies absconded (Ellis et al. 2003b). Other factors may have played a role in the absconding of the Cape control and treatment colonies (colony disturbance, nectar dearth, declining quality of the nest, etc.), because some control colonies absconded without any exposure to nest parasites (Hepburn and Radloff 1998, Ellis et al. 2003b). However, the data suggest that European colonies abscond in response to heavy beetle infestation (Ellis et al. 2003b).

Strangely, by the end of the experiment, it appeared that adult beetles migrated from the non-absconding European treatment colonies but not from the non-absconding Cape treatment colonies (Ellis et al. 2003b). Over half of the beetles introduced into the European treatment colonies were never recovered (Ellis et al. 2003b). It is possible that these beetles were host seeking, but no beetles were found in the control colonies (Ellis et al. 2003b). It may be that Cape honey bees are more efficient at imprisoning and guarding adult beetles than European honey bees (Ellis et al. 2003b).

At high infestation levels, European honey bees uncapped their own brood, aborting and cannibalizing in preparation for absconding (Ellis et al. 2003b). European control colonies did not exhibit this behavior (Ellis et al. 2003b). Cape honey bees were less affected by high small hive beetle populations and did not experience the same decline in brood (Ellis et al. 2003b). In general, absconding Cape colonies leave little to no food stores and capped brood behind and are

very effective in conserving colony resources (Spiewok et al. 2006c). Cape colonies were able to cope with the adult beetles, but it is unclear exactly how Cape honey bees prevent beetle damage over European colonies (Ellis 2004).

*Beetle Behavior.*

After an absconding event, food stores and brood are left behind (Hepburn and Radloff 1998). Such a glut of unprotected food provides adult beetles with plenty of nutrition for vast numbers of eggs and larvae. However, beetles may also be in competition with ants and other nest scavengers (Neumann and Elzen 2004).

During a European honey bee absconding event, five to 10 beetles were seen leaving the hive with the bee cluster (Ellis 2004). The bees landed on the ground some 15 meters away from the hive (Ellis 2004). When an empty hive box was placed next to the cluster, a beetle was seen entering the new box with the bees (Ellis 2004). It appeared that the beetle had been present in the bee cluste; however, the beetle may have joined the cluster while the bees were on the ground and not necessarily followed the bees from the hive (Ellis 2004). Beetles were also observed leaving with bees when a second colony absconded (Ellis 2004). This swarm was not successfully captured, so it is unknown whether beetles were truly present in the cluster (Ellis 2004). Adult beetles are efficient at finding colonies through olfactory cues, so it is possible that adult beetles simply follow the cluster by smell, tracking the bees to their new location.

**Alternate Hosts.**

*Bumble Bee Colonies.*

Unfortunately, honey bees are not the only bees to be affected by the small hive beetle. Several studies have shown that the bumble bee *Bombus impatiens* is also susceptible to depredation by these beetles, even though this bee species does not occur in the small hive

beetle's natural range (Ambrose et al. 2000, Spiewok and Neumann 2006b, Hoffmann et al. 2008). Because honey bees and bumble bees share similar traits, such as wax comb construction and nectar/pollen collection, these beetles are able to successfully utilize bumble bee colonies (Ambrose et al. 2000). Other bee species with these traits are also likely at risk (Ambrose et al. 2000, Hoffmann et al. 2008).

Initial studies confirmed that beetles could infest commercial bumble bee colonies under laboratory conditions (Ambrose et al. 2000). Infested colonies exhibited more comb damage and also a higher proportion of dead bees (Ambrose et al. 2000). Among these dead bees, many were no longer intact, suggesting that small hive beetles scavenged on the corpses (Ambrose et al. 2000). In these artificially-infested bumble bee colonies, beetles were able to reproduce and successfully complete an entire life cycle (Ambrose et al. 2000).

Later studies found that small hive beetles could survive and reproduce in *B. impatiens* colonies in the field (Spiewok and Neumann 2006b, Hoffmann et al. 2008). These studies involved artificially-infested field colonies, so it is still unknown whether beetles will naturally infest bumble bee colonies (Hoffmann et al. 2008). It is possible that wild bumble bees are susceptible to small hive beetle infestation (Spiewok and Neumann 2006b, Hoffmann et al. 2008).

Small hive beetles are not native to the U.S., so native bumble bees have never encountered them until now. Still, bumble bee workers display a range of defensive behaviors in reaction to these beetles and their various life stages (Hoffmann et al. 2008). Bumble bees are efficient at removing unprotected beetle eggs, even more so than honey bees within the same time frame of 50 minutes (Hoffmann et al. 2008). The workers will consume the beetle eggs, jettison larvae from the colony, and kill larvae by stinging them (Hoffmann et al. 2008).

Suazo et al. (2003) found that adult beetles are highly attracted to volatiles from honey bee workers, freshly collected pollen, unripe honey, and slumgum. Spiewok and Neumann (2006b) found that beetles were also attracted to adult bumble bees and pollen collected from bumble bee colonies (Spiewok and Neumann 2006b). Male beetles were more attracted to bumble bee odors while female beetles were more attracted to honey bee odors (Spiewok and Neumann 2006b). The data also suggest that bumble bee colonies are more attractive to these beetles than similarly sized honey bee colonies (Hoffmann et al. 2008). Because adult beetles do not preferentially choose honey bees over bumble bees, bumble bees are likely to serve as alternate hosts (Hoffmann et al. 2008). However, it is difficult to predict the damage that small hive beetles might inflict upon native bumble bees without more research. With some bumble bee species already in decline, an additional stressor could prove to be detrimental.

*Stingless Bees.*

While there are no documented cases of small hive beetles invading stingless bee colonies, Anne Dollin has personally observed beetles in the nests of *Trigona carbonaria* (Greco et al. 2010). In addition, Mark Greco has observed heat-stressed *T. carbonaria* colonies succumbing to small hive beetle infestation (Greco et al. 2010).

While honey bees encapsulate beetles in propolis, these stingless bees "mummify" beetles with batumen, an analogous substance composed of cerumen and resin and sometimes plant matter and mud (Michener 1974, Greco et al. 2010). Stingless bees will constantly harass an adult beetle to keep the beetle in its defensive posture (Greco et al. 2010). While the beetle is being harassed, other bees work to coat the beetle's elytra and legs with batumen (Greco et al. 2010). Mummified beetles do not require any further attention and will eventually die from starvation (Greco et al. 2010). A single stingless bee cannot mummify a beetle alone; multiple

individuals are needed to mummify a beetle in much the same way as it requires multiple honey bee workers to encapsulate a hive intruder (Greco et al. 2010).

Mummification appears to be more effective in dealing with adult beetles than encapsulation (Greco et al. 2010). Encapsulated beetles can potentially escape and require the constant attention of guard bees (Neumann et al. 2001b, Ellis et al. 2003e). Mummified beetles cannot escape (Greco et al. 2010). The worker bees are then free to carry out other tasks, as it is not necessary to guard mummified adult beetles (Greco et al. 2010).

**Control.**

*Chemical.*

Chemical control methods were the first to be tested against the small hive beetle in the United States. The aim was to control the destructive pest quickly and efficiently and to conduct research on alternative control methods once the pest was at tolerable levels. Once these beetles were detected, the USDA immediately began testing chemicals (Elzen et al. 1998). Three pyrethroids and two organophosphates were effective, but a formamidine insecticide, another organophosphate, and a neurotransmitter agonist were not (Elzen et al. 1998). Product names were not released in this initial lab study by the USDA.

Preliminary experiments examined plastic strips impregnated with various chemicals (Elzen et al. 1999b). Pyrethroids were found to be highly effective, as were some organophosphates (Elzen et al. 1999b). However, in field trials, pyrethroid Apistan strips provided no control of adults or larvae (Elzen et al. 1999b). The most promising organophosphate in the field was coumaphos (Elzen et al. 1999b). Coumaphos (10%) strips were stapled to corrugated cardboard on the bottom boards (Elzen et al. 199a, 1999b). It was found to be highly attractive as a refuge and killed all adults that came into contact with the strip (Elzen et

al. 1999b). Larvae were not affected since they usually remain in the comb; only those that fell potentially encountered the trap (Elzen et al. 1999b). While pyrethroids were not effective in the hive environment, they were effective as ground drenches (Elzen et al. 1999b, Baxter et al. 2000). Permethrin (40%) applied at 5 ml per gram of water to the soil surface killed nearly all burrowed life stages and emerging adults (Baxter et al. 2000).

Specially designed refuge traps with fipronil inserts have been shown to be effective against adult beetles (Levot 2008). The case of the trap is made of a two piece plastic shell which holds a fipronol-treated cardboard insert (Levot 2008). These traps are specially designed to allow only beetle entry; worker bees are too large to pass through and thus do not come into contact with the chemical (Levot 2008). Once beetles are inside the trap, they contact the fipronil-treated insert and die (Levot 2008). Interestingly enough, adult beetles do not only take refuge in these traps to avoid aggressive or patrolling worker bees. Even when there were no workers present on the comb, beetles continued to hide in the traps (Levot 2008). A single trap placed on the bottom board of beetle-infested hives resulted in 62% adult beetle mortality in six weeks (Levot 2008). Worker bees appeared unaffected by the fipronil traps (Levot 2008). Fipronil residues in honey did not exceed 1 $\mu g\ kg^{-1}$ after using the traps for a month (Levot 2008).

Organic acids were shown to have great inhibitory effects on yeast growth in hives with some minor effects on small hive beetles (Schäfer et al. 2009). Because organic acids are often used to control *V. destructor* and *Galleria mellonella*, they may also provide a secondary benefit by hindering yeast growth and thus minimizing the fermentation of honey (Schäfer et al. 2009). When applied at the same rates as would be used for controlling these other pests, formic and acetic acid prevented yeast growth from occurring on malt-agar plates (Schäfer et al. 2009). Lactic and oxalic acid only partially inhibited yeast growth (Schäfer et al. 2009). Adult beetle

mortality was higher on acetic acid treatments than the controls (Schäfer et al. 2009). Formic acid treatments resulted in lower larval infestation but not significant adult mortality (Schäfer et al. 2009). The formic acid may have negatively affected the reproductive potential of the adults, resulting in lowered larval presence (Schäfer et al. 2009). Because organic acids evaporate in hives, these treatments should be further tested for efficacy under different environmental conditions (Schäfer et al. 2009).

Because adult small hive beetles are able to be transported via package bees, some initial work was done to see if it would be feasible to control beetles in packages (Baxter et al. 1999). Checkmite +® strips (Bayer Corp.) stapled to the top of packages (so that the strip hung down into the package) provided the best control (over 94% beetle mortality) out of four different Checkmite +® treatment placements (Baxter et al. 1999). However, it was found that many smaller-sized adults would just leave the package because the 10-mesh wire was large enough for them to pass through (Baxter et al. 1999). Only the larger beetles would remain in the package with the bees (Baxter et al. 1999). Once bees are shaken into a package, most beetles immediately make their escape (Baxter et al. 1999). Baxter et al. (1999) thus believe it would be more efficient to control small hive beetles in the colony, rather than in individual packages, to ensure that bee packages are beetle-free.

There are many downsides to using chemicals in the hive to control small hive beetles (Lacey et al. 2001). With excessive usage, these beetles are likely to develop pesticide resistance (Lacey et al. 2001) in much the same way that select populations of *Varroa* mites have developed resistance to coumaphos and fluvalinate (Pettis 2004). Chemicals can also pose a threat to humans, honey bees, and domestic animals (Lacey et al. 2001). Among some of the environmental concerns, chemicals can contaminate ground water, taint honey bee products, and

affect non-target species (Lacey et al. 2001). Studies have shown that some of these chemicals can seep into bee products (Wallner 1999). Several varroacides leave detectable traces in beeswax which can then be transferred to honey or syrup (Wallner 1999, Kochansky et al. 2001). Bromopropylate, coumaphos, and fluvalinate are among the most commonly detected chemicals (Wallner 1999). Years and years of treatment result in the buildup of these residues in bee products (Wallner 1999). These fat-soluable residues are moved around the hive on the legs and bodies of the worker bees (Wallner 1999). Combs with coumaphos or other chemicals should be used for the brood chamber and not for honey to limit potential chemical contamination (Kochansky et al. 2001).

*Mechanical and Cultural.*

*Physically Removing Beetles.*

Beekeepers may lessen small hive beetle damage by physically killing them with hive tools or by removing them with hand-held vacuum devices (Hood 2010). However, these methods are generally regarded as inefficient. Killing beetles by hand is time-consuming (Hood 2010). Keeping the hive open for this period of time may be distressing to the bees. In addition, physically removing small hive beetles would only be suited for hobbyist beekeepers (Hood 2010).

Hood (2010) reports that at least 80% of beetles can be removed from a colony through physical means. This particular procedure requires two people with a lot of time. First, the queen is found and placed in a cage for safety (Hood 2010). Second, the hive frames are shaken over a light-colored plastic table with someone standing by to aspirate or vacuum any dislodged beetles (Hood 2010). The other pieces of the hive are gently bumped against the table to knock out any

hiding beetles (Hood 2010). Because some beetles manage to escape or fly away, this procedure is not 100% effective. However, it can result in significantly lowered populations (Hood 2010).

*Sanitation Practices.*

Small hive beetles are troublesome in the honey house, especially in honey houses that occur next to infested apiaries (Eischen et al. 1999b, Hood 2004, Spiewok et al. 2007). The microclimate of the honey house is suitable for beetle reproduction and development (Somerville 2003). Because honey supers may not be extracted immediately, these unprotected sources of honey can draw in beetles and result in an infestation in four to five days (Eischen et al. 1999b, Elzen et al. 2000c, Somerville 2003). Adults that are attracted to the honey house may have already had a protein meal, and these beetles will be able to lay eggs immediately. Even if adults do not have access to protein, they can survive on honey for more than five months and become a lingering problem (Lundie 1940, Ellis et al. 2002b). However, there are likely to be dead bees, old cocoons, and perhaps some pollen on the combs which may provide enough protein to sustain the adults and larvae. Because the adults and larvae can quickly ruin stored honey and combs, it is important to extract honey promptly, move out combs, and clean up any wax cappings, slumgum, and honey spillage (Eischen et al. 1999b, Hood 2000). Honey should be extracted from their frames within one or two days of being removed from the colony (Somerville 2003). Alternatively, honey supers can be kept in cold storage if they cannot be extracted within a few days (Elzen et al. 2000c). Honey houses with low small hive beetle infestations are associated with proper sanitation practices (Spiewok et al. 2007).

Ellis et al. (2002b) showed that adults can survive on empty brood comb for up to 50 days. Leftover pollen and other debris were probably present, allowing the beetles to survive for an extended period of time. It is important that beekeeping equipment is properly stored (Ellis et

al. 2002b). Cold storage rooms will prevent adult and larval activity and development (Somerville 2003). It is necessary to fumigate used material or material gathered from dead or weak colonies to prevent infesting new colonies or re-infesting old ones (Somerville 2003). Freezing used material for 24 hours may be a suitable alternative to fumigation (Somerville 2003).

Pollen traps can be another potential problem. They are usually left in hives for long periods of time. The pollen is scraped off of forager bees and lands into a catch box that cannot be accessed by the workers (Ellis et al. 2002b). This source of protein could be utilized by beetles, and it has been shown that beetles have high reproductive potential on pollen diets (Schmolke 1974, Ellis et al. 2002b). Emptying the contents of the pollen trap on a regular basis will prevent beetles from long-term access to these unprotected food stores (Ellis et al. 2002b).

Spiewok and Neumann (2006a) discovered several developmental stages of the small hive beetle in the debris of the bottom board, surviving in the absence of honey, pollen, and bee brood. Although small hive beetle reproduction in this sort of situation would be fairly low, it is important to allow workers access to all parts of the hive so that they can remove any debris that could support beetle development (Spiewok and Neumann 2006a). Strong colonies should keep the bottom board debris-free; weaker colonies that allow debris build-up may need to be re-queened (Spiewok and Neumann 2006a).

*Modified Hive Entrances.*

There have been several studies relating to modifying the hive entrance in order to reduce small hive beetle infestations (Ellis et al. 2003g, Hood and Miller 2005). It was thought that a reduced entrance may allow for more efficient guarding by guard bees, making it more difficult for adult beetles to invade (Ellis et al. 2003g). Another thought was that a modified entrance

might prevent wandering larvae from leaving the hive to pupate (Hood and Taber 2000). Results have not been promising, and the use of modified hive entrances appears to be associated with harmful side effects (Ellis et al. 2003g, Hood and Miller 2005).

Ellis et al. (2003g) found inconsistent results when narrowing the hive entrance with a 3.8-cm polyvinyl chloride pipe. Some colonies with a reduced entrance showed less infestation than colonies with standard entrances, but other colonies with reduced entrances showed no difference when compared to these control colonies (Ellis et al. 2003g). Some negative side effects were noted. Reduced hive entrances resulted in a decrease in brood production, excess bottom board debris, and water buildup from poor drainage (Ellis et al. 2003g). Screened bottom boards can lessen the severity of some of these effects but cannot mitigate them entirely (Ellis et al. 2003g). Pipe entrances did not impair the colony's ability to thermoregulate internal hive temperatures (Ellis et al. 2003g). Pollen storage was not affected by 3.8-cm pipe entrances as long as there was a screened bottom board (Ellis et al. 2003g). Smaller entrances, such as the 1.9-cm pipe entrance that was also examined, resulted in significantly less pollen storage (Ellis et al. 2003g).

Frake et al. (2009) found that wooden entrance reducers (2.2 cm width, 1.3 cm height) resulted in fewer small hive beetles when tested in Russian and Italian colonies. Unlike in the study conducted by Ellis et al. (2003g), no brood loss was observed and pollen storage did not seem to be affected (Frake et al. 2009).

Hood and Miller (2005) examined the effects of a 3.5-cm upper pipe entrance placed 20 cm above the hive bottom. Standard entrances were sealed off with a block of wood (Hood and Miller 2005). This upper hive entrance did not appear to impede small hive beetle ingress, as colonies with upper hive entrances did not result in reduced beetle infestation (Hood and Miller

2005). The use of this modified entrance also resulted in a significant reduction of brood (37.8% decrease) (Hood and Miller 2005).

Hood and Miller (2005) do not believe that a narrowed entrance will increase guarding efficiency or prevent wandering larvae from leaving the hive. They reason that most adult beetles enter honey bee colonies before or after dusk (Schmolke 1974), a time when guard bees are less active and protective (Hood and Miller 2005). Because reduced entrances result in a reduction of bee brood, there would be less guard bees available to defend the entrance against intruders (Hood and Miller 2005). It is unknown whether these entrances actually prevent larvae from escaping the hive. Inspection of the soil underneath hives with pipe entrances revealed that small hive beetle larvae were present (Hood and Miller 2005). However, these larvae may have wandered from adjacent colonies (Hood and Miller 2005). Neumann and Härtel (2004) noted that honey-coated beetle larvae were able to scale vertical surfaces because they were covered in a slimy honey coat. I have witnessed pollen-honey-coated larvae scaling the sides of plastic food containers.

*Non-chemical Substances and Desiccants.*

Some non-chemical substances have also been tested against small hive beetle adults and larvae. These include diatomaceous earth, slaked lime, and powdered limestone (Richards et al. 2005, Buchholz et al. 2009). If these substances are applied to the soil, non-target organisms are likely to be affected (Buchholz et al. 2009). Their effect on honey bees and bee products should also be examined, since some of these substances can be used within the hive (Buchholz et al. 2009).

Diatomaceous earth is composed of the skeletons of diatoms. The substance readily crumbles into an abrasive powder. This powder has been used for control against other insects

(Buchholz et al. 2009). It absorbs lipids from the waxy insect epicuticle, causing dehydration that may result in death (Buchholz et al. 2009). Naturally, results vary depending on the insect species, the lipid-binding capacity of the diatomaceous earth, and the relative humidity of the environment (Mewis and Ulrich 1999, Buchholz et al. 2009). Diatomaceous earth is less effective in humid environments because it absorbs water, becomes saturated, and is no longer effective in absorbing lipids from insect epicuticle (Buchholz et al. 2009). As a result, some formulations have been modified to become hydrophobic and can be used in more humid environments than regular formulations (Ulrichs et al. 2006).

Richards et al. (2005) found that regular formulations of diatomaceous earth resulted in very low mortality (2.56 ± 2.88%) of small hive beetle pupae. In fact, mortality from diatomaceous earth was not significantly different from the controls. Pupae exposed to diatomaceous earth in combination with *Aspergillus niger*, a generalist fungus, showed similarly low mortality (Richards et al. 2005). Microscopic investigations showed that the diatomaceous earth rarely caused lacerations deep enough to allow fungal spore access (Richards et al. 2005). In addition, the moisture content of the pupation chambers may have offset the dehydrating properties of the diatomaceous earth (Richards et al. 2005).

Buchholz et al. (2009) tested several hydrophobic formulations of diatomaceous earth (Fossil Shield®) against small hive beetle adults and wandering larvae. The three products that were tested were FS 90.0, FS 95.0, and FS 90.0s (Buchholz et al. 2009). FS 90.0 and FS 95.0 are considerably less hydrophobic than FS 90.0s (Buchholz et al. 2009). High and low dosages of FS 95.0 and FS 90.0 had no effect on beetle emergence (Buchholz et al. 2009). However, high larval mortality resulted from exposure to FS 90.0s in the soil (Buchholz et al. 2009). When placed in traps (a plastic tray with a grill), FS 90.0s also effectively controlled adult beetles (Buchholz et

al. 2009). In treated colonies, 50-65% of the adult beetle population died within 48 hours (Buchholz et al. 2009). Buchholz et al. (2009) noted that the diatomaceous earth was ventilated by the workers during thermoregulation of the colony. This resulted in the spread of FS 90.0s and dusty conditions throughout the hive. It is unknown whether products like FS 90.0s will harm the bees and contaminate honey or other bee products (Buchholz et al. 2009). Buchholz et al. (2009) suggest using traps that would contain the diatomaceous earth without allowing it to become airborne in the colony.

Slaked lime is a dry, hydrophilic substance (Buchholz et al. 2009). When applied to the soil, it alters soil pH and moisture levels which may negatively affect pupating beetles (Buchholz et al. 2009). When applied as a layer in the soil, slaked lime was not effective in controlling wandering larvae (Buchholz et al. 2009). The larvae simply burrowed beneath the lime layer and pupated in the untreated soil below (Buchholz et al. 2009). Slaked lime mixed into autoclaved soil was consistently effective in high doses (10 to 15 g per 100 g of soil) (Buchholz et al. 2009). Higher levels of slaked lime may sufficiently dehydrate the soil and cause mortality in the larvae and pupae (Buchholz et al. 2009). Mortality also occurred with low doses of slaked lime in non-autoclaved soil, but this may have been due to increased pathogen activity from the altered pH (Buchholz et al. 2009). Further studies should be conducted to determine how exactly slaked lime results in larval mortality.

Powdered limestone did not negatively affect adult beetle emergence (Buchholz et al. 2009). Mortality from the treatments (powdered limestone applied at 5, 10, and 15 g per 100 g of soil) did not differ significantly from the controls (Buchholz et al. 2009). In fact, adults appeared to have slightly greater emergence success in soil with powdered limestone (Buchholz et al.

2009). The powdered limestone appeared to enhance the soil, creating a more suitable pupation environment for the beetles (Buchholz et al. 2009).

*Baiting, Trapping, and Monitoring.*

Pollen dough (a mixture of pollen and honey) has been shown to be an effective bait after it has been conditioned through beetle feeding or inoculated with the yeast *K. ohmeri* (Arbogast et al. 2007, Torto et al. 2007c). Both methods of modifying the pollen dough were attractive to adult beetles (Torto et al. 2007c). However, beetle responses varied with the amount of conditioning of the pollen dough (Torto et al. 2007c). Beetles showed a stronger response to pollen dough that had been fed on by adult beetles for three and seven days (Torto et al. 2007c). Pollen dough conditioned for only a single day or for 14 days was only weakly attractive (Torto et al. 2007c). As adults feed on pollen dough, volatiles are released. These volatiles are produced by yeasts (including *K. ohmeri*) during fermentation (Torto et al. 2007c). Torto et al. (2007c) suggest that volatile production peaks between three to seven days, and it is during this time that the pollen dough will be most attractive to beetles. In response to yeast-inoculated pollen dough, male beetles were far more responsive than females (Torto et al. 2007c). No response differences were recorded between the sexes to unmodified pollen dough (Torto et al. 2007c). Frass volatiles appeared to reduce the attractiveness of pollen dough (Torto et al. 2007c). Pollen dough conditioned for 14 days showed high levels of 2-phenylethanol due to the increasing amount of beetle frass (Torto et al. 2007c).

Because attractive baits can be produced using *K. ohmeri*, beetle colonies do not need to be maintained for this purpose (Torto et al. 2007b). Torto et al. (2007b) explains that the yeast can be mass produced and may be more effective than keeping beetle colonies or developing synthetic attractants. In-hive traps baited with conditioned pollen dough caught significantly

more beetles than unbaited traps (Torto et al. 2007b). Also, baited traps caught more beetles when placed on the bottom board than when placed near the top of the hive (Torto et al. 2007b). This is in line with Lundie's (1940) finding that beetles tend to congregate on the bottom board. In fact, these baited bottom board traps nearly eradicated adult beetle populations in bee colonies in Florida (Torto et al. 2007b). Baited bottom board traps with a soapy solution showed promising results as a method of monitoring small hive beetle infestations in hives (Torto et al. 2007b).

Arbogast et al. (2007, 2009) tested conditioned and yeast-inoculated pollen dough in flight traps. Traps that were located in full shade caught more beetles than traps located in partial shade or full sunlight (Arbogast et al. 2007, 2009). No beetles were attracted to the control traps baited with water (Arbogast et al. 2007). Catch frequency decreased as traps were placed further and further from honey bee colonies (Arbogast et al. 2009). These data suggest that traps placed near apiaries in full shade may provide useful monitoring information for beekeepers (Arbogast et al. 2007, 2009).

Nolan and Hood (2008) examined two attractants: yeast-based (with *K. ohmeri*) and cider vinegar. Both attractants were attractive to small hive beetles, except that the yeast attractant was more effective during the warmer months (Nolan and Hood 2008). The cider vinegar attractant would evaporate within a few weeks during warm months (Nolan and Hood 2008). On the other hand, the yeast attractant required more preparation, as it requires mixing and conditioning before use (Nolan and Hood 2008). Neither of these attractants negatively affected honey bee brood production (Nolan and Hood 2008). Nolan and Hood (2008) also warn of using overly potent attractants which may lure in beetles from outside of the apiary.

De Guzman et al. (2011) utilized yeast-inoculated pollen dough in traps of different heights and colors. Little is known about how beetles respond to visual cues (de Guzman et al. 2011). Traps were made of either black or white PVC pipe and hung at three heights (46 cm, 1 m, and 3 m) within apiaries (de Guzman et al. 2011). While the number of beetles attracted to these traps was relatively low, it was still apparent that adults preferred white traps over black traps (de Guzman et al. 2011). Traps hung at a height of 46 cm (same height as hive entrances) attracted the most beetles (de Guzman et al. 2011). Because trap capture was fairly poor, this trap design requires some modification before it can be used as a method of monitoring beetle populations within apiaries (de Guzman et al. 2011).

Not all traps require an attractive bait. In a study by Schäfer et al. (2008), a refuge trap was shown to be effective in monitoring adult beetle populations within hives. Schäfer et al. (2008) used sheets of corrugated plastic to create a trap with numerous narrow tunnels. The original prototype had tunnels large enough for bees to enter, resulting in only one adult beetle being captured (Schäfer et al. 2008). A smaller-tunneled trap which excluded worker bees proved to be far superior to the original design (Schäfer et al. 2008). These unbaited refuge traps were located on the bottom board of the hive (Schäfer et al. 2008). Traps were left for two nights in the hive before being collected (Schäfer et al. 2008). Because the number of trapped beetles correlated with the number of beetles in the hive, Schäfer et al. (2008) suggest that this trapping method will help beekeepers quantify their beetle infestation levels. Further studies should be conducted, but these preliminary results show great potential.

*Biological.*

Relatively little work has been done on the biological control of small hive beetles. Lundie (1940) and Schmolke (1974) failed to identify any biological control agents in the small

hive beetle's natural range. When raising these beetles, Lundie (1940) noted that a small percentage of pupae succumbed to an unidentified fungus. Whether the fungus was a generalist or specialist is unknown. He also noted that *Microbracon brevicornis* (Hymenoptera: Braconidae) was occasionally found on wax moth larvae. In the laboratory environment, these wasps were observed stinging beetle larvae and feeding on the hemolymph from the puncture wounds. However, no wasps were successfully reared from beetle larvae. All beetle larvae died within two to three days of being stung. In addition, Schmolke (1974) never found any parasites or diseases in wild-caught or captive-raised specimens.

In Kenya, the generalist ant predator *Pheidole megacephala* was found predating on beetle pupae (Torto et al. 2010). These ants were significantly more active during the dry season (Torto et al. 2010). Beetle larvae also tended to burrow deeper during the dry season, perhaps in an effort to find moist soil and/or to avoid coming into contact with these ants (Torto et al. 2010). Larvae that were close to the soil surface were readily preyed upon (Torto et al. 2010). While this ant is useful in controlling other African pests, it prefers other foods over insects (Torto et al. 2010).

*Fungi.*

Entomopathogenic fungi have been responsible for the rearing failures in many previous experiments (Lundie 1940, Ellis et al. 2004a, Mürrle and Neumann 2004). Fungi can cause epizootics in their host species and regulate their populations (Lacey et al. 2001). Hyphomycetes (such as *Metarhizium*, *Beauveria*, and *Hirsutella*) attack a wide range of insect pest species (Lacey et al. 2001). Members of this group have been used to control whiteflies, aphids, thrips, termites, grasshoppers, and beetles (Lacey et al. 2001). In general, Hyphomycetes can be easily mass produced on artificial media and can be kept for long periods of time and are still viable for

later use (Lacey et al 2001). Entomophthoralean fungi, on the other hand, are fairly difficult to produce and have very short shelf lives (Lacey et al. 2001).

A preliminary study on the susceptibility of small hive beetle pupae to fungal pathogens showed that pupal mortality was higher (about 32% versus 4% in control) when larvae were exposed to generalist fungi (*Aspergillus flavus and A. niger*) during the post-feeding, wandering phase (Ellis et al. 2004b). The mortality of larvae which fed on fungus-infected food did not differ significantly from larvae which fed on control food (Ellis et al. 2004b). Dead pupae were examined and the fungi were confirmed to be present on the cadavers (Ellis et al. 2004). However, the death of these pupae may have been caused by another microorganism and then subsequently became infected with a fungus, so results should be analyzed with caution (Ellis et al. 2004b).

Another study further examined the effects of *A. flavus* and *A. niger* on small hive beetle pupae (Richards et al. 2005). In this study, mortality by the fungi was confirmed. *A. flavus* resulted in higher mortality (38.44 ± 3.75%) than *A. niger* (5.11 ± 2.67%) (Richards et al. 2005). Diatomaceous earth alone caused very low mortality (2.56 ± 2.88%), as did diatomaceous earth in conjunction with *A. niger* (3.11 ± 3.69%) (Richards et al. 2005). Pupal mortality caused by *A. niger* alone, diatomaceous earth alone, and *A. niger* plus diatomaceous earth did not differ significantly from the controls (Richards et al. 2005). *A. flavus* with diatomaceous earth resulted in high mortality (46.33 ± 14.15%) but did not differ significantly from *A. flavus* alone (Richards et al. 2005).

Even if *A. flavus* was effective in the field against small hive beetles, the risks of using this fungus would likely make it an inappropriate mycoinsecticide (Richards et al. 2005). First, the aflatoxin created by *A. flavus* is not host-specific (Kendrick 1992). It can affect insects other

than small hive beetles, as well as plants, livestock, companion animals, and humans (Richards et al. 2005). It can potentially cause hepatic disease in livestock and liver cancer in humans (Kendrick 1992). Ingesting aflatoxin-contamined honey can also cause aflatoxicosis (Kendrick 1992). In addition, *A. flavus* can also cause stonebrood in honey bees (Schmid-Hempel 1998). While stonebrood is generally a disease of minor importance, the concentrated application of *A. flavus* could cause severe stonebrood problems (Richards et al. 2005).

An unidentified fungus from a previous study (Mürrle and Neumann 2004) was isolated and identified using molecular techniques (Mürrle et al. 2006). This fungus was found to be closely related to *Metarhizium anisopliae* var. *anisopliae* strain FI-203 (Mürrle et al. 2006). This was the first fungal pathogen to be found on small hive beetles in their native range (Mürrle et al. 2006). Small hive beetles showed significantly increased mortality in the presence of this fungus (28.00 ± 16.43%) (Mürrle et al. 2006). Three other isolates of entomopathogenic fungi from Africa were also examined: *Beauveria bassiana*, *Hirsutella illustris*, and *M. anisopliae* (Mürrle et al. 2006). Significant mortality was observed with *B. bassiana* (74.00 ± 8.94%) (Mürrle et al. 2006). There was very little mortality with *H. illustris* (2.00 ± 4.47%) and *M. anisopliae* (12.00 ± 8.37%); these two treatments were not significantly different from the controls (Mürrle et al. 2006).

*Nematodes.*

Entomopathogenic nematodes are another possible biological control agent for small hive beetles. Members of the families Steinernematidae and Heterorhabditidae are well-known as insect specialists (Georgis and Manweiler 1994). Both of these families have a mutualistic relationship with *Xenorhabdus* bacteria (Kaya and Gaugler 1993). Once the nematode has entered a host, the bacteria aid in killing the host by septicemia, usually within 48 hours (Kaya

and Gaugler 1993). These nematodes attack a wide range of insect pests which encompass most of the insect orders (Georgis and Manweiler 1994). The infective juveniles (=IJs) share characteristics of both insect parasitoids and microbial pathogens (Kaya and Gaugler 1993). Like parasitoids, they are highly mobile, kill their hosts, and have a numerical response (Kaya and Gaugler 1993). Like pathogens, they are virulent and reproduce quickly (Kaya and Gaugler 1993). In general, nematodes are specific to their insect host and cause no harm to non-target organisms such as plants, vertebrates, and other invertebrates (Kaya and Gaugler 1993). In the United States, nematodes are exempt from registration (Kaya and Gaugler 1993). In the United Kingdom, only native nematodes can be utilized (Kaya and Gaugler 1993). Nematodes are easy to mass rear, formulate, and apply (Georgis and Manweiler 1994). These nematode biopesticides can provide control that is on par with chemical applications (Georgis and Gaugler 1991). Georgis and Manweiler (1994) report that there are 13 species of *Steinernema* and four species of *Heterohabditis*. Glazer et al. (1999) found that Heterorhabditids were effective against sap beetles in date palm orchards. This suggests that species of Heterorhabditidae may perform well against small hive beetles.

While nematodes are often compatible with chemical applications, it has been shown that the fungus *B. bassiana* cannot compete with entomopathogenic nematodes in the same host (Barbercheck and Kaya 1990). The *Xenorhabdus* bacteria secrete an antibiotic which kills the fungus (Kaya and Gaugler 1993). The fungus can still be successful if it can infect a host a few days before nematode infection occurs (Kaya and Gaugler 1993). These two agents are able to partially coexist, as nematodes avoid fungus-infected hosts (Barbercheck and Kaya 1991a). These agents used in combination result in higher mortality than if used alone (Barbercheck and Kaya 1991b).

Three commercially available nematodes were tested against wandering beetle larvae: *Heterorhabditis megidis* HO I strain (LC50: 164 IJs/larva), *Steinernema carpocapsae* All strain (LC50: 204 IJs/larva), and *S. riobrave* Texas strain (LC50: 157 IJs/larva) (Cabanillas and Elzen 2006). Cabanillas and Elzen (2006) suggest that greater mortality may occur if nematodes were employed against the pupal stage rather than the larval stage. An actively moving larva is a more difficult target to infect than a sedentary pupa that remains motionless in the soil for several weeks (Cabanillas and Elzen 2006).

In a study conducted by Ellis et al. (2010), it was shown that *S. riobrave* Rio strain, *H. bacteriophora* Oswego strain, and *H. indica* were all effective against small hive beetle pupae and wandering larvae. However, beetles were only susceptible when subjected to high concentrations of infective juveniles (>200 IJs/larvae, 50 IJs/$cm^2$) (Glazer et al. 1999, Cabanillas and Elzen 2006, Ellis et al. 2010). Similar data was found in Glazer et al. (1999); sap beetles were considerably less susceptible to low concentrations of infective juveniles. *S. riobrave* 7-12 strain and *H. indica* controlled beetle larvae for 19 weeks after one soil inoculation in generational persistence bioassays (Ellis et al. 2010). Both of these nematodes caused 76 to 94% mortality in beetle pupae (Ellis et al. 2010). In a field bioassay, these two nematodes caused 88 to 100% mortality in pupating beetles (Ellis et al. 2010). The continuing flow of wandering larvae into the soil allows for a constant food supply for the nematodes, which may explain how they could persist for so long from a single application (Ellis et al. 2010). Should small hive beetle larvae stop entering the soil for an extended period of time, another nematode application may be needed (Ellis et al. 2010). It is possible that nematodes can be used as part of an integrated approach to maintain small hive beetles at tolerable levels (Ellis et al. 2010). However, in these previous studies, only commercially available nematodes were tested. To date

(and to my knowledge), no surveys have been conducted to explore the nematodes that infect small hive beetles in their current and introduced range.

*Bacteria.*

The well-known *Bacillus thuringiensis* has been shown to be effective against members of Lepidoptera, Coleoptera, and Diptera (Lacey et al. 2001). This bacterium produces proteinaceous endotoxins which are highly host specific (Lacey et al. 2001). Non-target organisms are very rarely at risk and even closely related species are usually left unaffected (Höfte and Whiteley 1989, Lacey et al. 2001). The bacteria are ingested and release their endotoxins into the gut of their insect host which results in the death of the insect (Lacey et al. 2001). One study examined three strains of *B. thuringiensis* against small hive beetles: var. aizawai (B401), var. kurstaki (Novodor), and var. tenebrionis (Jackpot) (Buchholz et al. 2006). However, none of these strains affected the number of wandering larvae that were produced (Buchholz et al. 2006). This supports the current data that *B. thuringiensis* is very selective, and that at least these commercially available strains show no significant effect on small hive beetles (Buchholz et al. 2006). Martin and Travers (1989) state that there is a high chance of finding *B. thuringiensis* in the soil. It is possible that there are strains that affect small hive beetles in Africa, but so far there has been little work done in the beetle's natural range.

*Other Control Agents.*

Other possible biological control agents have yet to be identified. The work that has been done thus far does not accurately reflect control agents in the small hive beetle's natural range; rather, generalist fungi and commercially available nematodes are the only agents that have been investigated. While it is important to determine if readily-accessible control agents are effective against these beetles, more work needs to be conducted on determining natural enemies in the

beetle's endemic range. To date, no specialist nematodes, bacteria, viruses, protozoans, or microsporidia (now classified as fungi) have been found on beetles in Africa. Surveys of small hive beetles from Africa (and also beetles in introduced areas) may show infection by microorganisms. It is imperative that we examine beetles for the often overlooked control agents like bacteria, protozoa, and viruses which may have great effects on beetle mortality. Examining the soil where beetles pupate may also reveal pathogens that may cause chronic illness, reduced fecundity, or mortality to small hive beetles.

**References.**


Ambrose, J. T., M. S. Stanghellini, and D. I. Hopkins. 2000. A scientific note on the threat of small hive beetles (*Aethina tumida* Murray) to bumble bee (*Bombus* spp.) colonies in the United States. Apidologie 31: 455-456.

(Anon). 2003. Small hive beetle found in Australia. Am. Bee J. 143(1): 14.

Arbogast, R. T., B. Torto, D. V. Engelsdorp, and P. E. A. Teal. 2007. An effective trap and bait combination for monitoring the small hive beetle, *Aethina tumida* (Coleoptera: Nitidulidae). Fla. Entomol. 90(2): 404-406.

Arbogast, R. T., B. Torto, and P. E. A. Teal. 2009a. Monitoring the small hive beetle *Aethina tumida* (Coleoptera: Nitidulidae) with baited flight traps: effect of distance from bee hives and shade on the numbers of beetles captured. Fla. Entomol. 92(1): 165-166.

Arbogast, R. T., B. Torto, S. Willms, and P. E. A. Teal. 2009b. Trophic habits of *Aethina tumida* (Coleoptera: Nitidulidae): their adaptive significance and relevance to dispersal. Environ. Entomol. 38(3): 561-568.

Arbogast, R.T., B. Torto, and P. E. A. Teal. 2010. Potential for population growth of the small hive beetle *Aethina tumida* (Coleoptera: Nitidulidae) on diets of pollen dough and oranges. Fla. Entomol. 93(2): 224-230.

Barbercheck, M. E., and H. K. Kaya. 1990. Interactions between *Beauveria bassiana* and the entomogenous nematodes, *Steinernema feltiae* and *Heterorhabditis heliothidis*. J. Invertebr. Pathol. 55: 225-234.

Barbercheck, M. E., and H. K. Kaya. 1991a. Effect of host condition and soil texture on host finding by the entomogenous nematodes *Heterohabditis bacteriophora* (Rhabditida: Heterohabditidae) and *Steinernema carpocapsaeI* (Rhabditida: Steinernematidae). Environ. Entomol. 20: 528-589.

Barbercheck, M. E., and H. K. Kaya. 1991b. Competitive interactions between entomopathogenic nematodes and *Beauveria bassiana* (Deuteromycotina: Hyphomycetes) in soilborne larvae of *Sporoptera exigua* (Lepidoptera: Noctuidae). Environ. Entomol. 20: 707-712.

Bartelt, R. J. 1999. Pheromones of non-lepidopteran insects associated with agricultural plants, CABI publishing, Wallingford, pp. 69-89.

Baxter, J. R., P. J. Elzen, D. Westervelt, D. Causey, C. Randall, F. A. Eischen, and W. T. Wilson. 1999. Control of the small hive beetle, *Aethina tumida* in package bees. Am. Bee J. 139(10): 792-793.

Baxter, J. R., P. J. Elzen, and W. T. Wilson. 2000. Gardstar 40% EC (Permethrin) efficacy trials as a ground drench for the control of small hive beetle around honey bee colonies. TEKTRAN, USDA Agricultural Research Service; 1 pp.

Benda, N. D., D. Boucias, B. Torto, and P. Teal. 2008. Detection and characterization of *Kodamaea* ohmeri associated with small hive beetle *Aethina tumida* infesting honey bee hives. J. Apic. Res. and Bee World 47(3): 194-201.

Brown, M. A., H. M. Thompson, and M. H. Bew. 2002. Risks to UK beekeeping from the parasitic mite *Tropilaelaps clarae* and the small hive beetle, *Aethina tumida*. Bee World 83(4): 151-164.

Buchholz, S., P. Neumann, K. Merkel, and H. R. Hepburn. 2006. Evaluation of *Bacillus thuringiensis* Berliner as an alternative control of small hive beetles, *Aethina tumida* Murray (Coleoptera: Nitidulidae). J. Pest. Sci. 79: 251-254.

Buchholz, S., M. O. Schäfer, S. Spiewok, J. S. Pettis, M. Duncan, W. Ritter, R. Spooner-Hart, and P. Neumann. 2008. Alternative food sources of *Aethina tumida* (Coleoptera: Nitidulidae). J. Apic. Res. and Bee World 47(3): 202-209.

Buchholz, S., K. Merkel, S. Spiewok, J. S. Pettis, M. Duncan, R. Spooner-Hart, C. Ulrichs, W. Ritter, and P. Neumann. 2009. Alternative control of *Aethina tumida* Murray (Coleoptera: Nitidulidae) with lime and diatomaceous earth. Apidologie 40: 535-548.

Cabanillas, H. E., and P. J. Elzen. 2006. Infectivity of entomopathogenic nematodes (Steinernematidae and Heterorhabditidae) against the small hive beetle *Aethina tumida* (Coleoptera: Nitidulidae). J. Apic. Res. and Bee World 45(1): 49-50.

Caron, D. M., A. Park, J. Hubner, R. Mitchell, and I. B. Smith. 2001. Small hive beetle in the Mid-Atlantic states. Am. Bee J. 141(1): 776-777.

Connor, Larry. 2011. Wipe out!: the big island in crisis. Bee Culture 139(4): 55-60.

Cuthbertson, A. G. S., J. J. Mathers, L. F. Blackburn, M. E. Wakefield, L. E. Collins, W. Luo, and M. A. Brown. 2008. Maintaining *Aethina tumida* (Coleoptera: Nitidulidae) under quarantine laboratory conditions in the UK and preliminary observations on its behavior. J. Apic. Res. and Bee World 47(3): 192-193.

de Guzman, L., and A. M. Frake. 2007. Temperature affects *Aethina tumida* (Coleoptera: Nitidulidae) development. J. Apic. Res. 46(2): 88-93.

de Guzman, L. I., A. M. Frake, and T. E. Rinderer. 2008. Detection and removal of brood infested with eggs and larvae of small hive beetles (*Aethina tumida* Murray) by Russian honey bees. J. Apic. Res. and Bee World 47(3): 216-221.

de Guzman, L. I., J. A. Prudente, T. E. Rinderer, A. M. Frake, and H. Tubbs. 2009. Population of small hive beetles (*Aethina tumida* Murray) in two apiaries having different soil textures in Mississippi. Science of Bee Culture 1(1): 4-8.

de Guzman, L. I., A. M. Frake, and T. E. Rinderer. 2010. Seasonal population dynamics of small hive beetles, *Aethina tumida* Murray, on the south-eastern USA. J. Apic. Res. and Bee World 49(2): 186-191.

de Guzman, L. I., A. M. Frake, T. E. Rinderer, and R. T. Arbogast. 2011. Effect of height and color on the efficiency of pole traps for *Aethina tumida* (Coleoptera: Nitidulidae). J. Econ. Entomol. 104(1): 26-31.

Delaplane, K. S., J. D. Ellis, and W. M. Hood. 2010. A test for interactions between *Varroa destructor* (Acari: Varroidae) and *Aethina tumida* (Coleoptera: Nitidulidae) in colonies of honey bees (Hymenoptera: Apidae). Ann. Entomol. Soc. Am. 103(5): 711-715.

Dixon, D., and R. Lafrenière. 2002. The small hive beetle in Manitoba. Manitoba Beekeeper, Fall 2002.

Eischen, F. A., D. Westervelt, and C. Randall. 1999a. Does the small hive beetle have alternative food sources? Am. Bee J. 139: 125.

Eischen, F. A., D. Westervelt, and J. Baxter. 1999b. Small hive beetles in the honey house! Am. Bee. J. 139(12): 934-935.

Ellis, J. D. 2002. Life behind bars: why honey bees feed small hive beetles. Am. Bee J. 142(4): 267-269.

Ellis, J. D., K. S. Delaplane, and W. M. Hood. 2002a. Small hive beetle (*Aethina tumida* Murray) weight, gross biometry, and sex proportion at three locations in the southeastern United States. Am. Bee J. 142(7): 520-522.

Ellis, J. D., P. Neumann, R. Hepburn, and P. J. Elzen. 2002b. Longevity and reproductive success of *Aethina tumida* (Coleoptera: Nitidulidae) fed different natural diets. J. Econ. Entomol. 95(5): 902-907.

Ellis, J. D., C. W. W. Pirk, H. R. Hepburn, G. Kastberger, and P. J. Elzen. 2002c. Small hive beetles survive in honeybee prisons by behavioral mimicry. Naturwissenschaften 89: 326-328.

Ellis, J. D., R. Hepburn, K. S. Delaplane, and P. J. Elzen. 2003a. A scientific note on small hive beetle (*Aethina tumida*) oviposition behaviour during European (*Apis mellifera*) honey bee clustering and absconding events. J. Apic. Res. 42(1-2): 47-48.

Ellis, J.D., R. Hepburn, K. S. Delaplane, P. Neumann, and P. J. Elzen. 2003b. The effects of adult small hive beetles, *Aethina tumida* (Coleoptera: Nitidulidae), on nests and flight activity of Cape and European honey bees (*Apis mellifera*). Apidologie 34: 399-408.

Ellis, J. D., A. J. Holland, R. Hepburn, P. Neumann, and P. J. Elzen. 2003c. Cape (*Apis mellifera capensis*) and European (*Apis mellifera*) honey bee guard age and duration of small hive beetle (*Aethina tumida* Murray, Coleopera: Nitidulidae) prison guarding. J. Apic. Res. 43(3): 32-34.

Ellis, J. D., C. S. Richards, H. R. Hepburn, and P. J. Elzen. 2003d. Oviposition by small hive beetles elicits hygienic responses from Cape honeybees. Naturwissenschaften 90: 532-535.

Ellis, J. D., H. R. Hepburn, A. M. Ellis, and P. J. Elzen. 2003e. Social encapsulation of the small hive beetle (*Aethina tumida* Murray) by European honeybees (*Apis mellifera* L.). Insectes Soc. 50: 286-291.

Ellis, J. D., H. R. Hepburn, A. M. Ellis, and P. J. Elzen. 2003f. Prison construction and guarding behavior by European honeybees is dependent on inmate small hive beetle density. Naturwissenschaften 90: 382-384.

Ellis, J. D., K. S. Delaplane, R. Hepburn, and P. J. Elzen. 2003g. Efficacy of modified hive entrances and a bottom screen device for controlling *Aethina tumida* (Coleoptera: Nitidulidae) infestations in *Apis mellifera* (Hymenoptera: Apidae) colonies. J. Econ. Entomol. 96(6): 1647-1652.

Ellis, J. D. 2004. The ecology and control of small hive beetles (*Aethina tumida* Murray). Ph.D. dissertation, Rhodes University, Grahamstown, South Africa.

Ellis, J. D., R. Hepbern, B. Luckman, and P. J. Elzen. 2004a. Effects of soil type, moisture, and density on pupation success of *Aethina tumida* (Coleoptera: Nitidulidae). Environ. Entomol. 33(4): 794-798.

Ellis, J.D., I.H. Rong, M.P. Hill, H.R. Hepburn, P.J. Elzen. 2004b. The susceptibility of small hive beetle (*Aethina tumida* Murray) pupae to fungal pathogens. Am. Bee J. 144(6): 486-488.

Ellis, J. D., K. S. Delaplane, C. S. Richards, R. Hepburn, J. A. Berry, and P. J. Elzen. 2004c. Hygienic behavior of Cape and European *Apis mellifera* (Hymenoptera: Apidae) toward *Aethina tumida* (Coleoptera: Nitidulidae) eggs oviposited in sealed bee brood. Ann. Entomol. Soc. Am. 97(4): 860-864.

Ellis, J. D. 2005. Reviewing the confinement of small hive beetles (*Aethina tumida*) by western honey bees (*Apis mellifera*). Bee World 86(3): 56-62.

Ellis J. D., Munn P.A. 2005. The worldwide health status of honey bees. Bee World 86: 88-101.

Ellis, J. D., and K. S. Delaplane. 2008. Small hive beetle (*Aethina tumida*) oviposition behavior in sealed brood cells with notes on the removal of the cell contents by European honey bees (*Apis mellifera*). J. Apic. Res. and Bee World 47(3): 210-215.

Ellis, A., and K. S. Delaplane. 2008. Effects of nest invaders on honey bee (*Apis mellifera*) pollination efficacy. Agric. Ecosyst. Environ. 127: 201-206.

Ellis, J. D., K. S. Delaplane, A. Cline, and J. V. McHugh. 2008. The association of multiple sap beetle species (Coleoptera: Nitidulidae) with western honey bee (*Apis mellifera*) colonies in North America. J. Apic. Res. and Bee World 47(3): 188-198.

Ellis, J. D., and P. A. Munn. 2005. The worldwide health status of honey bees. Bee World 86: 88-101.

Ellis, J. D., S. Spiewok, K. S. Delaplane, S. Buchholz, P. Neumann, and W. L. Tedders. 2010. Susceptibility of *Aethina tumida* (Coleoptera: Nitidulidae) larvae and pupae to entomopathogenic nematodes. J. Econ. Entomol. 103(1): 1-9.

El-Niweiri, M. A. A., M. S. El-Sarrag, and P. Neumann. 2008. Filling the Sudan gap: the northernmost natural distribution limit of small hive beetles. J. Apic. Res. and Bee World 47(3): 184-185.

Elzen, P. J., J. R. Baxter, D. Westervelt, and R. Rivera. 1998. Small hive beetle control USDA initial lab study results. Bee Culture 126(10): 19-20.

Elzen, P. J., J. R. Baxter, D. Westervelt, C. Randall, K. S. Delaplane, L. Cutts, and W. T. Wilson. 1999a. Field control and biology studies of a new pest species, *Aethina tumida* Murray (Coleoptera, Nitidulidae), attacking European honey bees in the Western Hemisphere. Apidologie 30: 361-366.

Elzen, P. J., J. R. Baxter, D. Westervelt, C. Randall, L. Cutts, W. Wilson, F. A. Eischen, K. S. Delaplane, and D. I. Hopkins. 1999b. Status of the small hive beetle in the US. Bee Culture 127: 28-29.

Elzen P. J., J. R. Baxter, P. Neumann, A. J. Solbrig, C. W. W. Pirk, W. Hoffman, and H. R. Hepburn. 2000a. Observations on the small hive beetle in South Africa. Am. Bee J. 140(4): 304.

Elzen, P. J., J. R. Baxter, D. Westervelt, C. Randall, and W. T. Wilson. 2000b. A scientific note on observations of the small hive beetle, *Aethina tumida* Murray (Coleoptera, Nitidulidae), in Florida, USA. Apidologie 31: 593-594.

Elzen, P. J., J. R. Baxter, and M. Allsopp. 2000c. Small hive beetle experiences in South Africa. Bee Culture 128(4): 56.

Elzen, P. J., J. R. Baxter, P. Neumann, A. Solbrig, C. Pirk, H. R. Hepburn, D. Westervelt, and C. Randall. 2001. Behavior of African and European subspecies of *Apis mellifera* toward the small hive beetle, *Aethina tumida*. J. Apic. Res. 40(1): 40-41.

Elzen, P. J., D. Westervelt, D. Causey, J. Ellis, H. R. Hepburn, and P. Neumann. 2002. Method of application of Tylosin, an antibiotic for American foulbrood control, with effects on small hive beetle (Coleoptera: Nitidulidae) populations. J. Econ. Entomol. 95(6): 1119-1122.

Evans, J. D., J. S. Pettis, and H. Shimanuki. 2000. Mitochondrial DNA relationships in an emergent pest of honey bees: *Aethina tumida* (Coleoptera: Nitidulidae) from the United States and Africa. Ann. Entomol. Soc. Am. 93(3): 415-420.

Evans, J. D., J. S. Pettis, W. M. Hood, and H. Shimanuki. 2003. Tracking an invasive honey bee pest: mitochondrial DNA variation in North American small hive beetles. Apidologie 34: 103-109.

Evans, B. 2011. Small hive beetle infestation (*Aethina tumida*), Canada (follow-up report no. 4). OIE. World Animal Health Information Database, (http://web.oie.int/wahis/public.php?page=single_report&pop=1&reportid=10300).

Eyer, M., Y. P. Chen, M. O. Schäfer, J. Pettis, and P. Neumann. 2009a. Small hive beetle, *Aethina tumida*, as a potential biological vector of honeybee viruses. Apidologie 40: 419-428.

Eyer, M., Y. P. Chen, M. O. Schäfer, J. S. Pettis, and P. Neumann. 2009b. Honey bee sacbrood virus infects adult small hive beetles, *Aethina tumida* (Coleoptera: Nitidulidae). J. Apic. Res. and Bee World 48(4): 296-297.

Fletcher, M. J., and L. G. Cook. 2002. Small hive beetle. Agnote DAI-288. NSW Agriculture. pp 3.

Frake, A. M., L. I. de Guzman, T. E. Rinderer. 2009. Comparative resistance of Russian and Italian honey bees (Hymenoptera: Apidae) to small hive beetles (Coleoptera: Nitidulidae). J. Econ. Entomol. 102(1): 13-19.

Georgis, R., and R. Gaugler. 1991. Predictability in biological control using entomopathogenic nematodes. J. Econ. Entomol. 84: 713-720.

Georgis, R., and S. A. Manweiler. 1994. Entomopathogenic nematodes: a developing biological control technology, pp. 63-94. In K. Evans (ed.), Agricultural Zoology Reviews, vol. 6. Intercept Ltd., Andover, UK.

Gillespie, P., J. Staples, C. King, M. J. Fletcher, and B. C. Dominiak. 2003. Small hive beetle, *Aethina tumida* (Murray) (Coleoptera: Nitidulidae) in New South Wales. Gen. Appl. Ent. 32: 5-7.

Glazer, I., L. Salame, S. Goldenberg, and D. Blumberg. 1999. Susceptibility of sap beetles (Coleoptera: Nitidulidae) to entomopathogenic nematodes. Biocontrol Sci. Tech. 9: 259-266.

Greco, M. K., D. Hoffmann, A. Dollin, M. Duncan, R. Spooner-Hart, P. Neumann. 2010. The alternate Pharaoh approach: stingless bees mummify beetle parasites alive. Naturwissenschaften 97: 319-323.

Haque, N. M. M., and G. W. Levot. 2005. An improved method of laboratory rearing the small hive beetle *Aethina tumida* Murray (Coleoptera: Nitidulidae). Gen. Appl. Entomol. 34: 29-31.

Hansen H., C.J. Brødsgaard. 1997. Der Verlauf der Amerikanischen (Bösartigen) Faulbrud in künstlich infizierten Völkern, Allg. Dtsch. Imkerztg. 3: 11-14.

Hassan, A. R., and P. Neumann. 2008. A survey for the small hive beetle in Egypt. J. Apic. Res. and Bee World 47(3): 186-187.

Hepburn, H. R., R. C. Cantrill, P. R. Thompson, and E. Kennedi. 1979. Metabolism of carbohydrate, lipid and protein during development of sealed worker brood of the African honeybee. J. Apic. Res. 18(1): 30-35.

Hepburn, H. R., and S. E. Radloff. 1998. Honeybees of Africa. Springer-Verlag, Berlin, New York.

Hoffmann, D., J. S. Pettis, and P. Neumann. 2008. Potential host shift of the small hive beetle (*Aethina tumida*) to bumblebee colonies (*Bombus impatiens*). Insectes Soc. 55: 153-162.

Höfte, H., and H. R. Whiteley. 1989. Insecticidal crystal proteins of Bacillus thuringiensis. Microbiol. Rev. 53: 242-255.

Hood, W. M. M. 2000. Overview of the small hive beetle, *Aethina tumida*, in North America. Bee World 81(3): 129-137.

Hood, W. M., and S. Taber. 2000. Search for European honey bees that show cleansing habits for removal of the small hive beetle, *Aethina tumida* Murray, in the USA, p. 905. *In* Proceedings, American Bee Research Conference, 4-5 August 2000, Salisbury, MD. Am. Bee J. Hamilton, IL.

Hood, W. M. M. 2004. The small hive beetle, *Aethina tumida*: a review. Bee World 85(3): 51-59.

Hood, W. M., and G. A. Miller. 2005. Evaluation of an upper hive entrance for control of *Aethina tumida* (Coleoptera: Nitidulidae) in colonies of honey bees (Hymenoptera: Apidae). J. Econ. Entomol. 98(6): 1791-1795.

Hood, M. 2010. IPM for the small hive beetle. Bee Culture 138(3): 31-33.

Kaya, H. K., and R. Gaugler. 1993. Entomopathogenic nematodes. Ann. Rev. Entomol. 38: 181-206.

Keller, J. J. 2002. Testing effects of alternative diets on reproduction rates of the small hive beetle, *Aethina tumida*. M.S. thesis, North Carolina State University, Raleigh.

Kendrick, B. 1992. The Fifth Kingdom, 2nd Ed. Mycologue Publication, Waterloo, Canada.

Kochansky, J., K. Wilzer, and M. Feldlaufer. 2001. Comparison of the transfer of coumaphos from beeswax into syrup and honey. Apidologie 32: 119-125.

Lacey, L. A., R. Frutos, H. K. Kaya, P. Vail. 2001. Insect pathogens as biological control agents: Do they have a future? Biol. Control 21: 230-248.

Levot, G. W. 2008. An insecticidal refuge trap to control adult small hive beetle, *Aethina tumida* Murray (Coleoptera: Nitidulidae) in honey bee colonies. J. Apic. Res. and Bee World 47(3): 222-228.

Lundie, A. E. 1940. The small hive beetle, *Aethina tumida*. Sci. Bull. 220, Union of South Africa, Dept Agric Forestry, 30p.

Lundie, A. E. 1952. The principle diseases and enemies of honey bees. S. Afr. Bee J. 27: 13-15.

Martin, P. A. W., and R. S. Travers. 1989. Worldwide abundance and distribution of *Bacillus thuringiensis* isolates. Appl. Environ. Microbiol. 55: 2437-2442.

Mewis, I., and C. Ulrichs. 1999. Wirkungsweise amopher Diatomeenerden auf vorratsschädliche Insekten: untersuchung der abrasive sowie sorptiven Effekte. Anz. Schädlingsk. 72: 113-121.

Michener, C. D. 1974. The Social Behavior of the Bees: A Comparative Study. Belknap Press of Harvard University Press, Cambridge, Massachusetts.

Mostafa, A. M., and R. N. Williams. 2002. New record of the small hive beetle in Egypt and notes on its distribution and control. Bee World 83(3): 99-108.

Murray, A. 1867. List of Coleoptera received from Old Calabar, on the west coast of Africa. The Annals and Magazine of Natural History, London 19: 176-177.

Mürrle, T., and P. Neumann. 2004. Mass production of small hive beetles (*Aethina tumida*, Coleoptera: Nitidulidae). J. Apic. Res. 43(2): 144-145.

Mürrle, T. M., P. Neumann, J. F. Dames, H. R. Hepburn, and M. P. Hill. 2006. Susceptibility of adult *Aethina tumida* (Coleoptera: Nitidulidae) to entomopathogenic fungi. J. Econ. Entomol. 99(1): 1-6.

Neumann, P., C. W. W. Pirk, R. Hepburn, P. J. Elzen, and J. R. Baxter. 2001a. Laboratory rearing of small hive beetles *Aethina tumida* (Coleoptera, Nitidulidae). J. Apic. Res. 40: 111-112.

Neumann, P., C. W. W. Pirk, H. R. Hepburn, A. J. Solbrig, F. L. W. Ratnieks, P. J. Elzen, and J. R. Baxter. 2001b. Social encapsulation of beetle parasites by Cape honeybee colonies (*Apis mellifera capensis* Esch.). Naturwissenschaften 88: 214-216.

Neumann, P., and P. J. Elzen. 2004. The biology of the small hive beetle (*Aethina tumida*, Coleoptera: Nitidulidae): gaps in our knowledge of an invasive species. Apidologie 35: 229-247.

Neumann, P., and S. Härtel. 2004. Removal of small hive beetle (*Aethina tumida*) eggs and larvae by African honeybee colonies (*Apis mellifera scutellata*). Apidologie 35: 31-36.

Neumann, P., and W. Ritter. 2004. A scientific note on the association of *Cychramus luteus* (Coleoptera: Nitidulidae) with honey bee (*Apis mellifera*) colonies. Apidologie 35: 665-666.

Neumann, P., and J. D. Ellis. 2008. The small hive beetle (*Aethina tumida* Murray, Coleoptera: Nitidulidae): distribution, biology and control of an invasive species. J. Apic Res. and Bee World 47(3): 181-183.

Nolan IV, M. P., and W. M. Hood. 2008. Comparison of two attractants to small hive beetles, *Aethina tumida*, in honey bee colonies. J. Apic. Res. and Bee World 47(3): 229-233.

Oldroyd, B. P., and P. Nanork. 2009. Conservation of Asian honey bees. Apidologie 40: 296-312.

Pettis, J. S., and H. Shimanuki. 2000. Observations on the small hive beetle, *Aethina tumida* Murray, in the United States. Am. Bee J. 140: 152-155.

Pettis, J. S. 2004. A scientific note on *Varroa destructor* resistance to coumaphos in the United States. Apidologie 35: 91-92.

Richards, C. S., M. P. Hill, and J. F. Dames. 2005. The susceptibility of small hive beetle (*Aethina tumida* Murray) pupae to *Aspergillus niger* (van Tiegem) and *A. flavus* (Link:Grey). Am. Bee J. 145(9): 748-751.

Roulston T.H., J.H. Cane, S.L. Buchmann. 2000. What governs protein content of pollen: Pollinator preferences, pollen-pistil interactions, or phylogeny? Ecol. Monog. 70(4): 617-643.

Schäfer, M. O., J. S. Pettis, W. Ritter, and P. Neumann. 2008. A scientific note on quantitative diagnosis of small hive beetles, *Aethina tumida*, in the field**.** Apidologie 39: 564-565.

Schäfer, M. O., W. Ritter, J. S. Pettis, P. E. A. Teal, and P. Neumann. 2009. Effects of organic acid treatments on small hive beetles, *Aethina tumida*, and the associated yeast *Kodamaea ohmeri*. J. Pest. Sci. 82: 283-287.

Schäfer, M. O., W. Ritter, J. S. Pettis, and P. Neumann. 2010a. Winter losses of honeybee colonies (Hymenoptera: Apidae): The role of infestations with *Aethina tumida* (Coleoptera: Nitidulidae) and *Varroa destructor* (Parasitiformes: Varroidae). J. Econ. Entomol. 103(1): 10-16.

Schäfer, M. O., W. Ritter, J. Pettis, and P. Neumann. 2010b. Small hive beetles, *Aethina tumida*, are vectors of *Paenibacillus larvae*. Apidologie 41: 14-20.

Schmid-Hempel, P. 1998. Parasites in Social Insects. Princeton University Press, Princeton, New Jersey.

Schmolke, M. D. 1974. A study of *Aethina tumida*: the small Hive Beetle, Project Report, University of Rhodesia, p. 178.

Solbrig, A. J. 2001. Interactions between the South African honeybee *Apis mellifera capensis* Esch. and the small hive beetle *Aethina tumida*. M.S. thesis, Freie Universität, Germany.

Somerville, D. 2002. Bees and drought. Honeybee News 6: 20-21.

Somerville, D. 2003. Study of the small hive beetle in the USA. Rural Industries Research and Development Corporation, Barton, Australian Capital Territory. 57 pp.

Spiewok, S., and P. Neumann. 2006a. Cryptic low-level reproduction of small hive beetles in honey bee colonies. J. Apic. Res. and Bee World 45(1): 47-48.

Spiewok, S., and P. Neumann. 2006b. Infestation of commercial bumblebee (*Bombus impatiens*) field colonies by small hive beetles (*Aethina tumida*). Ecol. Entomol. 31: 623-628.

Spiewok, S., P. Neumann, and H. R. Hepburn. 2006c. Preparation for disturbance-induced absconding of Cape honeybee colonies (*Apis mellifera capensis* Esch.). Insect Soc. 53: 27-31.

Spiewok, S., J. S. Pettis, M. Duncan, R. Spooner-Hart, D. Westervelt, and P. Neumann. 2007. Small hive beetle, *Aethina tumida*, populations I: Infestation levels of honeybee colonies, apiaries and regions. Apidologie 38: 595-605.

State of Hawai'i, Department of Agriculture. 2010. Another serious bee pest found in Hilo. (http://hawaii.gov/hdoa/news/2010-news-releases/another-serious-bee-pest-found-in-hilo/).

Suazo, A., B. Torto, P. E. A. Teal, and J. H. Tumlinson. 2003. Response of the small hive beetle (*Aethina tumida*) to honey bee (*Apis mellifera*) and beehive-produced volatiles. Apidologie 34: 525-533.

Suh, S. O., and M. Blackwell. 2005. Four new yeasts in the *Candida mesenterica* clade associated with basiodiocarp-feeding beetles. Mycologia 97(1): 167-177.

Swart, J. D., M. F. Johannsmeier, G. D. Tribe, and P. Kryger. 2001. Diseases and pests of honeybees, pp. 198-222. In M. F. Johannsmeier (ed.), Beekeeping in South Africa, 3rd Ed., Plant Protection Research Institute Handbook No. 14, Agricultural Res. Council of South Africa, Pretoria, South Africa.

Torto, B., D. G. Boucias, R. T. Arbogast, J. H. Tumlinson, and P. E. A. Teal. 2007a. Multitrophic interaction facilitates parasite-host relationship between an invasive beetle and the honey bee. Proc. Nat. Acad. Sci. USA 104(20): 8374-8378.

Torto, B., R. T. Arbogast, D. Engelsdorp, S. Willms, D. Purcell, D. Boucias, J. H. Tumlinson, and P. E. A. Teal. 2007b. Trapping of *Aethina tumida* (Coleoptera: Nitidulidae) from *Apis mellifera* L. (Hymenoptera: Apidae) colonies with an in-hive baited trap. Environ. Entomol. 36(5): 1018-1024.

Torto, A., R. T. Arbogast, H. Alborn, A. Suazo, D. Engelsdorp, D. Boucias, J. H. Tumlinson, and P. E. A. Teal. 2007c. Composition of volatiles from fermenting pollen dough and attractiveness to the small hive beetle *Aethina tumida*, a parasite of the honeybee *Apis mellifera*. Apidologie 38: 380-389.

Torto, B., A. T. Fombong, R. T. Arbogast, and P. E. A. Teal. 2010. Monitoring *Aethina tumida* (Coleoptera: Nitidulidae) with baited bottom board traps: occurrence and seasonal abundance in honey bee colonies in Kenya. Environ. Entomol. 39(6): 1731-1736.

Ulrichs, C., S. Entenmann, A. Goswami, and I. Mewis. 2006. Abrasive and hydrophilic/lipophilic effects of different inert dusts used as insecticide against the stored insect pest *Sitophilus granaries* L. Gesunde Pflanzen 58: 173-181.

Wallner, K. 1999. Varroacides and their residues in bee products. Apidologie 30: 235-248.

Weiss, M. R. 2006. Defecation behavior and ecology of insects. Ann. Rev. Entomol. 51: 635-661.

Wenning, C. J. 2001. Spread and threat of the small hive beetle. Am. Bee J. 141(9): 640-643.

Westervelt, D., D. Causey, P. Neumann, J. Ellis, and R. Hepburn. 2001. Grease patties worsen small hive beetle infestations. Am. Bee J. 141(11): 775.

White, R. E. 1983. A Field Guide to the Beetles of North America. Houghton Mifflin Company, Boston, New York.

White, B. 2003. Small hive beetle update. Australian Beekeeper May 2003, No. 11.

Winston, M. L. 1987. The Biology of the Honey Bee. Harvard University Press, Cambridge, Massachusetts.

Winston, M. L. 1992. The honey bee colony: life history, pp. 73-101. In J. M. Graham (Ed.), The Hive and the Honey Bee. Dadant and Sons, Hamilton, Illinois.

## Chapter 2: A Clean and Cost Effective Method of Rearing Small Hive Beetles (*Aethina tumida)* in the Laboratory


### Abstract.

The small hive beetle (SHB) is an invasive pest of honey bees that can be found in at least 31 states in the United States. Laboratory rearing of SHBs allows for immediate access to adults and immature stages without having to constantly collect them from infested bee colonies. Several rearing methods have already been published, but there is room for improvement with each of these methods. Our method uses inexpensive and readily available materials and does not require the collection and use of brood/honey comb. SHB life stages are kept separate, resulting in a cleaner rearing approach. Our method prevents reproduction from occurring unless it is required by the researcher. Eggs of known age can be collected whenever needed by using oviposition guides.


### Introduction.

The small hive beetle (Coleoptera: Nitidulidae, SHB) is an invasive pest of honey bee colonies in the United States (Elzen et al. 1999, Hood 2004). Within three years of its arrival, the SHB had spread into thirteen states (Hood 2004). By 2008, the beetle could be found in 31 states (Neumann and Elzen 2004, Neumann and Ellis 2008). Clearly, the SHB is a pest that is going to remain in the United States and, as such, efforts should be made to learn more about its biology, interactions with other organisms, disease vectoring capability, and vulnerabilities. In addition, countries without SHBs may wish to study these beetles under secure conditions in order to prepare for a potential invasion. To do so, it is important that SHBs can be mass produced in the laboratory so that researchers can have beetles available for study and experimentation. Since

SHBs naturally aggregate in honey bee colonies (Schmolke 1974) and have multiple generations per year (Lundie 1940, Schmolke 1974, Somerville 2003), they can be kept together in large laboratory colonies and bred whenever it is necessary to produce immature stages or more adults.

Several researchers have developed methods to rear SHBs in the laboratory (Neumann et al. 2001, Mürrle and Neumann 2004, Haque and Levot 2005). The earliest method was reported by Neumann et al. (2001) three years after the discovery of the SHB in the United States. While their method works, it is complicated and messy. It involves using a pine board with drilled holes full of water in order to keep pupation containers moist and to provide refugia for wandering stage larvae. In their method, adults and larvae were fed pieces of bee comb containing brood, honey, and pollen. Mürrle and Neumann (2004) expanded upon the methods of Neumann et al. (2001) but continued to use pieces of brood comb to feed SHBs. The method of Haque and Levot (2005) used peat in their pupation soil mixtures, a maintenance diet of loose sucrose crystals, and crumpled paper towels for refugia. While there are a few things that can be improved upon, Haque and Levot's (2005) method was a tremendous step forward in simplifying SHB rearing. Our objective was to develop a rearing method for SHBs that was less messy and could be created using readily available materials on a low budget.

**Materials and Methods.**

A colony of SHBs was established at the University of Arkansas Cralley-Warren Research Lab in Fayetteville, Arkansas. SHBs were obtained from an infested honey bee colony at the Arkansas Agriculture Research and Extension Center. To do this, the outer cover of the hive was positioned on the ground in direct sunlight with the inner surface facing skyward (Ed Levi, personal communication). Two supers were then placed on top of the outer cover. The supers were removed from the outer cover after 10 minutes. A pooter-style aspirator (BioQuip,

California) was used to collect adult SHB that had gathered beneath the supers on the outer cover to escape the sunlight. Sections of SHB larvae-infested comb were taken back to the lab. The larvae from the comb were reared to adulthood. The hive parts were then bagged and frozen for a week to kill any remaining adults and larvae. Approximately 30 adult beetles were collected as well as ca. 3,500 larvae.

Adult beetles were maintained in a rearing container (Rubbermaid® easyfind lids™, 40 cm width x 13 cm height x 28 cm depth, 9.5 L capacity). A mesh insert (2.5 x 2.5 cm) in the lid allowed for ventilation. The upper 10 cm of the container were coated with Insect-a-Slip Barrier (BioQuip, California) to prevent beetles from climbing the sides of the container. Damp paper towels were used to line the bottom of the rearing container to maintain humidity. Crumpled paper towels and corrugated cardboard pieces (stacked five high) were used as refugia. A small, shallow dish (5 cm diameter) contained honey which was always in ample supply. A layer of plastic mesh screen (25 µm mesh opening) was placed in the honey dish to prevent beetles from getting stuck in the honey and drowning. The rearing container was cleaned out every three to four weeks and new paper towels and cardboard were provided. Paper towels were moistened every three days with a spray bottle. The paper towels were kept moist but the water was not allowed to pool, in order to prevent drowning of SHBs. The rearing container was taken into a 4°C cold room when maintenance had to be performed in order to keep beetles from flying out.

SHB adults and larvae were maintained in an incubator at 30°C in complete darkness. Adults were fed only honey to prevent reproduction (Lundie 1940, Ellis et al. 2002). When larvae were required, a subset of adults were removed and fed pollen-honey diet (1:1 by weight) presented to them in a 60mm Petri dish. Plastic oviposition guides were provided. These guides were similar to those provided by Arbogast et al. (2009). The guides were made from plastic

hanging folder tabs (Fig. 1). Tabs were cut into three equal sections and their upper edge was stapled (Fig. 2). Thin plastic strips were cut from the excess plastic of the folder tab and were used to produce a small space for oviposition (Fig. 3).

Females began laying eggs two days to one week after being fed on pollen-honey diet. The egg-laden guides (Fig. 4) were moved to a larval rearing chamber (Mainstays™ Food storage set, 19 cm width x 7 cm height x 13 cm depth, 1.2 L capacity) with a 2.5 x 2.5 cm mesh insert in the lid for ventilation. Moist paper towels and a small dish (60 mm Petri dish) with pollen-honey diet were provided. The pollen-honey diet was checked daily and refilled as necessary so that the diet was always in ample supply. The oviposition guides were placed at the edge of the diet. Upon hatching, first instar larvae crawled out and made their way towards the diet. Within five to seven days, most of the larvae were mature and at the wandering stage looking for pupation sites.

Wandering stage larvae were moved to a pupation container (Mainstays™ Food storage set, 19 cm width x 18 height x 18 cm depth, 3.8 L capacity). The pupation container was filled with moist, autoclaved soil to allow for at least 10 cm of burrowing depth. Soil consisted of three parts Quikrete Premium Play Sand® No. 1113 to two parts Arkansas soil (see Table 1 for soil analysis report). A U.S. Standard no. 20 sieve was used to remove rocks, pebbles, and debris from the Arkansas soil prior to mixing and autoclaving. For moist soil, 100g of water was added for every 900g of soil (Arbogast et al. 2009). Adult beetles began to emerge three to four weeks later. Adults were removed from the container on a daily basis as soon as emergence began. Adults were aspirated from their pupation container while in a 4°C cold room to prevent flying. Aspirated adults were then added to the main colony while in the cold room.

**Results and Discussion.**

Several generations of small hive beetles were readily produced under these conditions. For example, when ample supplies of our pollen-honey mixture were provided, five females and five males produced 511 wandering larvae by the end of two weeks (Fig. 5). This shows how simple it is to produce large numbers of eggs, larvae, pupae, and adults of known age for experimental purposes using our method. Beetles were bred when required, and females produced eggs within one week of being fed a protein meal. More than enough larvae were produced; in some cases, there were so many larvae that some had to be frozen.

Although wandering larvae have been recorded to burrow deeper than 10 cm (Pettis and Shimanuki 2000, Mürrle and Neumann 2004), Pettis and Shimanuki (2000) reported that most small hive beetle pupae in Florida were found within the top 10 cm of soil. This was used to determine the depth of soil in the pupation container.

Initially, adult beetles were offered sucrose crystals as an energy source. According to Haque and Levot (2005), adult beetles can be maintained on "loose sucrose crystals" to prevent reproduction. In our study, the adult beetle population began to dwindle and the original 30 adult SHBs died within a month when offered only sucrose crystals. When adults were offered honey, they survived for upwards of five months. It is possible that adult beetles may fare better on a sucrose/water solution than on sucrose crystals, but this was not investigated. Alternatively, honey may provide small hive beetles with nutrients that cannot be found in a sucrose-only diet.

Our method of raising small hive beetles is cost effective and most of the components can be bought from local retail stores. Neumann et al. (2001) and Mürrle and Neumann (2004) used honey, pollen, and/or brood comb to feed their adult beetles. Because pollen or brood are present in the combs, female beetles will have access to protein sources for egg and larval development.

However, honey comb and brood comb are not readily available unless one has access to honey bee colonies, and using brood and honey comb to feed SHBs is messy. In addition, SHB reproduction is constantly occurring under these conditions and larvae and adults are mixed together. SHB larvae are much messier than adults and lead to increased maintenance of the colony when raised together; our improved method also avoids this situation. Neumann et al. (2001) recommended including bee brood in the larval diet whenever possible, but we found that SHB larvae develop into vigorous, full-sized adults when fed only pollen as their protein source (Ellis et al. 2002, N. Wright personal observation).

Cutherbertson et al. (2008) used two liter bottles for their pupation containers. A small, honey-baited container was screwed on to the top of a two liter bottle. Adults emerging from the soil fly into the screw-on container, allowing for easy removal of newly-emerged adults. If a cold room is not available to subdue flying beetles for aspiration, then this alternative method of capturing emerged beetles may be more desirable. While two liter bottles are inexpensive and easy to obtain, it may be difficult to fill with moist soil and also to clean, because of the small and narrow opening. For this reason, we prefer the use of a plastic container with a wide mouth for ease of filling and cleaning.

Haque and Levot (2005) provided crumpled paper towels as SHB refugia. We provided SHBs with crumpled paper towels and stacked corrugated cardboard. While SHBs were found among both types of refugia, SHBs seemed to prefer the second to lowest layer of the cardboard nearest to their food source. The bottom layer seemed too moist and the highest layer (stacked five high) seemed too dry for most beetles, but beetles could be found among every layer, just in lower numbers. SHBs were also abundant in the paper towels that lined the bottom of the

container, but rarely were they found in the crumpled paper towels when other refugia were provided.

Sand and peat composed the pupation soil in Haque and Levot's (2005) rearing method. Unless peat is readily available, native soils may be used. Ellis et al. (2004) found that pupation occurred equally well in loamy sand, silty clay, sandy loam, and clay loam, as long as the soil was moist. We used loamy Arkansas soil mixed with play sand with great success and very low mortality (<5%).

Several studies have shown that small hive beetles are multivoltine (Lundie 1940, Schmolke 1974, Somerville 2003). Lundie (1940) reported five generations per year in South Africa and Somerville (2003) reported six generations per year under moderate weather conditions in the United States. Through producing beetles for various experiments and to maintain a colony of young and healthy adults, we can confirm that these beetles can produce more than four generations per year.

**Summary.**

1. Rearing materials for this method are inexpensive and readily available.
2. Eggs, larvae, pupae, and adults can be easily reared and kept separately.
3. Adult SHBs only oviposit when eggs are needed by the researcher.
4. Eggs and neonates of known age can be easily collected whenever necessary.
5. No need for honey or brood comb from bee colonies, resulting in less mess.

**References.**


Arbogast, R. T., B. Torto, S. Willms, and P. E. A. Teal. 2009. Trophic habits of *Aethina tumida* (Coleoptera: Nitidulidae): their adaptive significance and relevance to dispersal. Environ. Entomol. 38(3): 561-568.

Cuthbertson, A. G. S., J. J. Mathers, L. F. Blackburn, M. E. Wakefield, L. E. Collins, W. Luo, and M. A. Brown. 2008. Maintaining *Aethina tumida* (Coleoptera: Nitidulidae) under quarantine laboratory conditions in the UK and preliminary observations on its behavior. J. Apic. Res. and Bee World 47(3): 192-193.

Ellis, J. D., P. Neumann, R. Hepburn, and P. J. Elzen. 2002. Longevity and reproductive success of *Aethina tumida* (Coleoptera: Nitidulidae) fed different natural diets. J. Econ. Entomol. 95(5): 902-907.

Ellis, J. D., R. Hepbern, B. Luckman, and P. J. Elzen. 2004. Effects of soil type, moisture, and density on pupation success of *Aethina tumida* (Coleoptera: Nitidulidae). Environ. Entomol. 33(4): 794-798.

Haque, N. M. M., and G. W. Levot. 2005. An improved method of laboratory rearing the small hive beetle *Aethina tumida* Murray (Coleoptera: Nitidulidae). Gen. Appl. Entomol. 34: 29-31.

Hood, W. M. M. 2004. The small hive beetle, *Aethina tumida*: a review. Bee World 85(3): 51-59.

Lundie, A. E. 1940. The small hive beetle, *Aethina tumida*. Sci. Bull. 220, Union of South Africa, Dept Agric Forestry, 30p.

Mürrle, T., and P. Neumann. 2004. Mass production of small hive beetles (*Aethina tumida*, Coleoptera: Nitidulidae). J. Apic. Res. 43(2): 144-145

Neumann, P., C. W. W. Pirk, R. Hepburn, P. J. Elzen, and J. R. Baxter. 2001. Laboratory rearing of small hive beetles *Aethina tumida* (Coleoptera, Nitidulidae). J. Apic. Res. 40: 111-112.

Neumann, P., and P. J. Elzen. 2004. The biology of the small hive beetle (*Aethina tumida*, Coleoptera: Nitidulidae): gaps in our knowledge of an invasive species. Apidologie 35: 229-247.

Neumann, P., and J. D. Ellis. 2008. The small hive beetle (*Aethina tumida* Murray, Coleoptera: Nitidulidae): distribution, biology and control of an invasive species. J. Apic Res. and Bee World 47(3): 181-183.

Pettis, J. S., and H. Shimanuki. 2000. Observations on the small hive beetle, *Aethina tumida* Murray, in the United States. Am. Bee J. 140: 152-155.

Schmolke, M. D. 1974. A study of *Aethina tumida*: the small Hive Beetle, Project Report, University of Rhodesia, p. 178.

Somerville, D. 2003. Study of the small hive beetle in the USA. Rural Industries Research and Development Corporation, Barton, Australian Capital Territory. 57 pp.

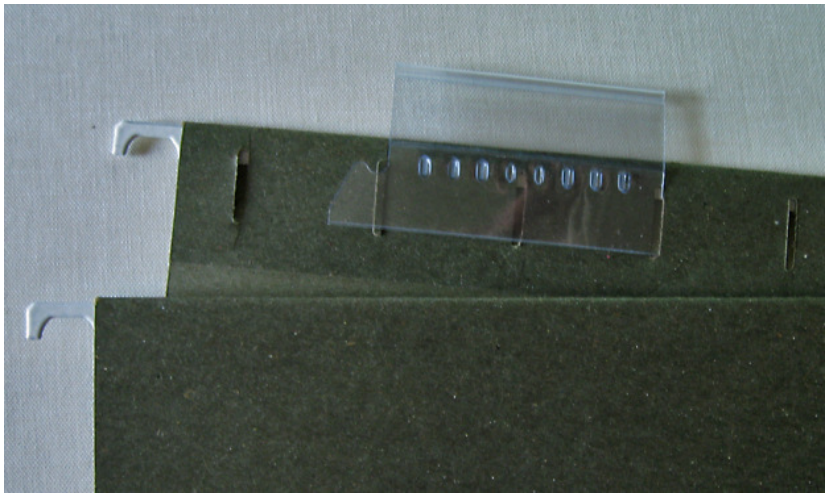

Fig. 1. Standard plastic hanging folder tab.

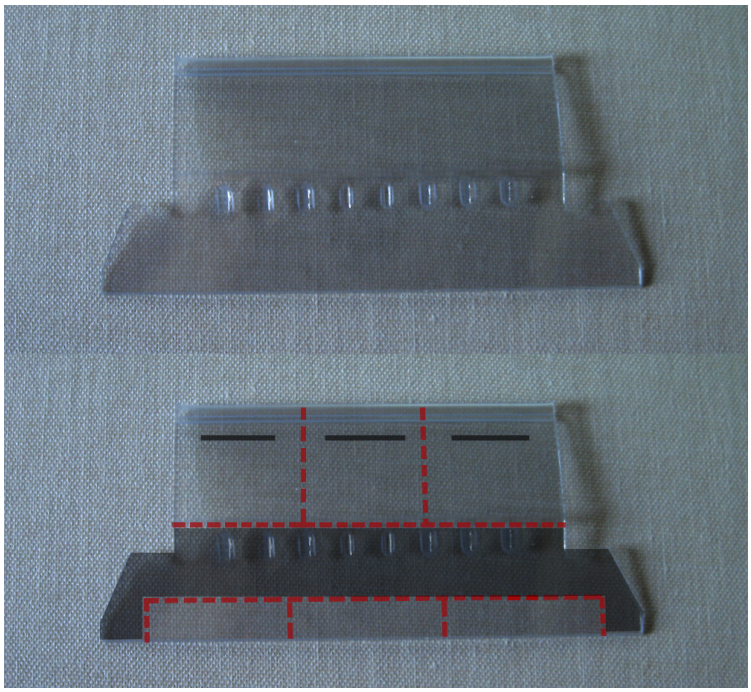

Fig. 2. Top, plastic hanging folder tab. Bottom, approximate cuts on a folder tab to produce three oviposition guides and six insertion strips. Only one strip is needed per oviposition guide. Red lines indicate cuts, black lines indicate staples. The shaded area was discarded.

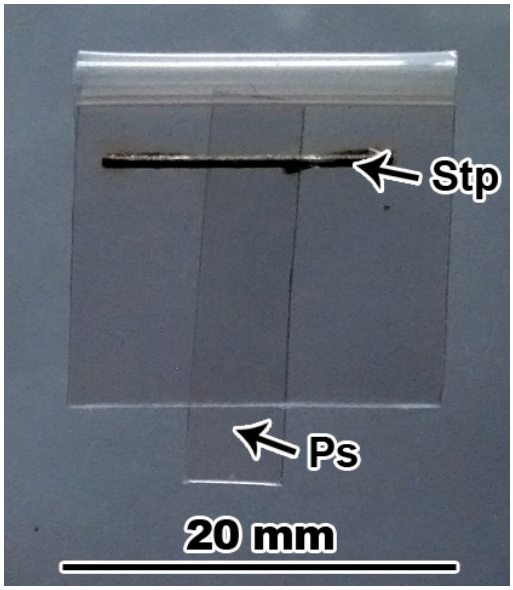


Fig 3. Oviposition guide made from a folder tab. *Stp* - staple, *Ps* - a single plastic strip insertion.

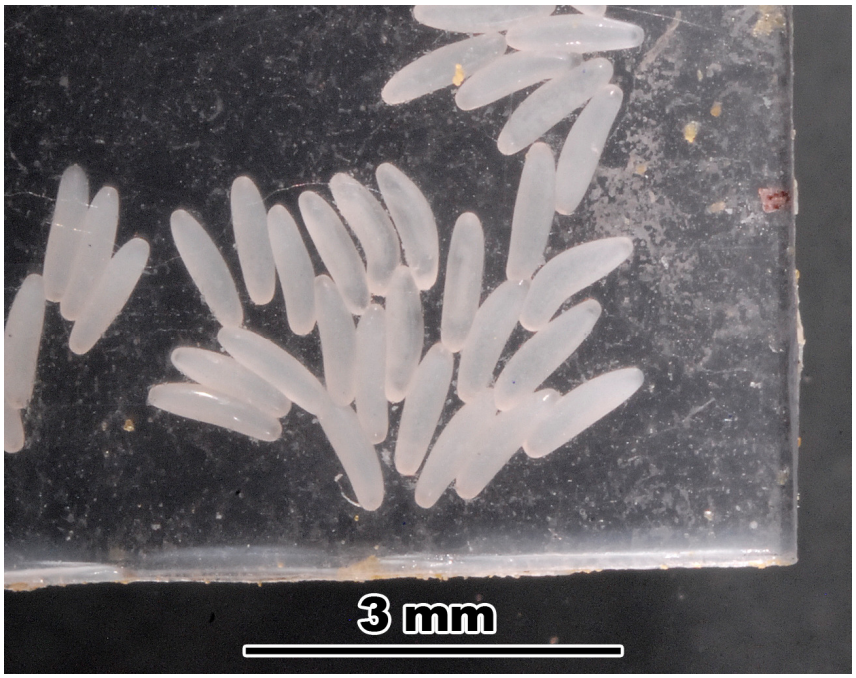


Fig. 4. Oviposition guide with SHB eggs.

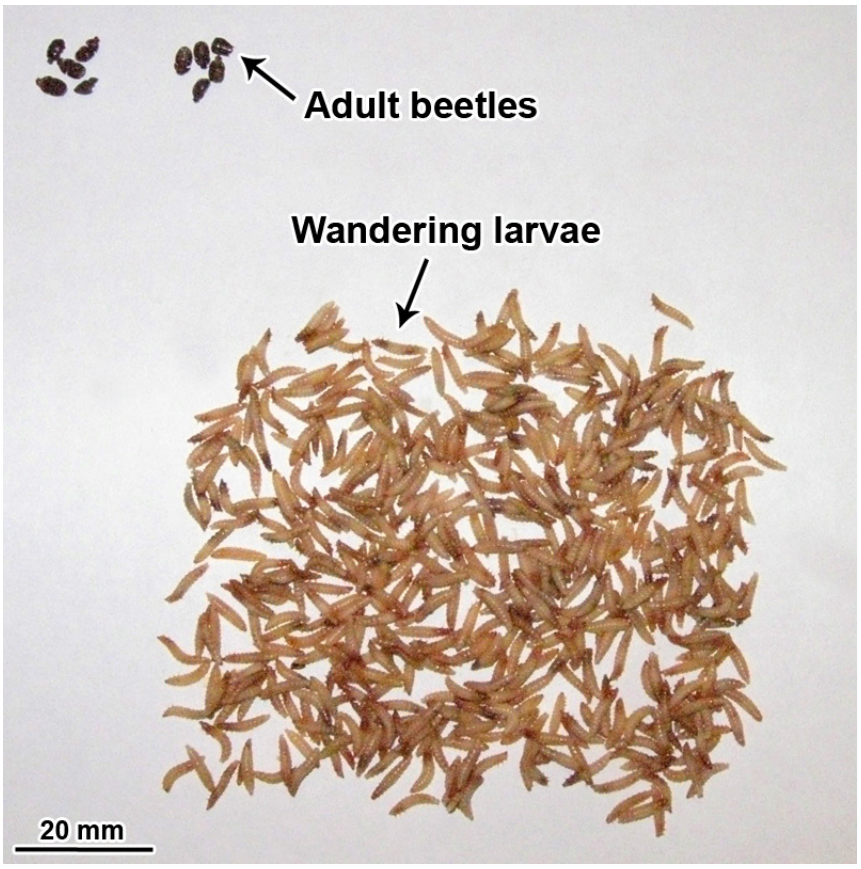


Fig. 5. Wandering larvae produced by five females and five males by the end of two weeks. Pollen-honey mixture was supplied on the first day and kept in amply supply for two weeks. The beetles were raised at room temperature. At the end of the two weeks, the adults and larvae were frozen and counted.

ARRIVED: 5-26-2011
LOGGED: 5-27-2011
OUT: 6-02-2011

AGRICULTURE DIAGNOSTIC LABORATORY
UNIVERSITY OF ARKANSAS, FAYETTEVILLE
RESEARCH SOIL SAMPLES

ADDRESS: ENTOMOLOGY

STUDY: SOIL SAMPLES
COST CENTER #:
PROCEDURES : pH,EC (1:2 soil/water ratio); Mehlich 3 (1:10 ratio) ANALYSIS BY SPECTRO ARCOS ICP

| | | | umhos/cm | mg/kg | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| LAB # | ID | pH | EC | P | K | Ca | Mg | S | Na | Fe | Mn | Zn | Cu | B |
| 11290 | BEETLE LOAM* | 8.03 | 135 | 11 | 54 | 1485 | 92 | 6 | 7 | 116 | 41 | 2.0 | 0.3 | 0.3 |

Table 1. Soil analysis of the Arkansas soil used for small hive beetle pupation (three parts sand to two parts Arkansas soil).

## Chapter 3: Dissection Techniques for Adult and Larval Small Hive Beetles (*Aethina tumida*) to Examine Internal Organs for Pathogens

### Introduction.

Small hive beetles (SHBs) are an invasive pest of honey bee colonies. Current control measures are mainly chemical or cultural. There are no host-specific biological control agents available for use on SHBs, although certain generalist fungi and commercially available nematodes have been shown to provide some control of the soil-dwelling stages of the SHB. There has only been one published article on the internal pathogenic microorganisms that infect these beetles and that was done on SHB in Arkansas. No investigations have been conducted on SHBs in their native range in Africa where pathogens are much more likely to be found. In addition, there have been no published studies on dissection techniques to examine the organs of adult and larval SHBs. The difficulty in dissecting SHBs lies in their very small size, their fragility, and their highly sclerotized exoskeleton. Fresh specimens are considerably easier to dissect, but fresh specimens are not always available. Adults SHBs preserved in 70% ethanol are brittle and prone to breaking at the pronotum when handled. Once the pronotum has broken away from the rest of the body, it becomes considerably more difficult to dissect the specimen.

After many attempts, we developed a reliable method for dissecting adult and larval SHBs which minimizes the loss of specimens, regardless of whether they are fresh or preserved. The objective of this paper is to present our findings on optimum dissection methods for examining *A. tumida* for pathogens.

**Materials and Methods.**

*Adult dissections.*

Adults and larvae were dissected using iris scissors, minuten pins (12 mm long, 0.20 mm diameter), and fine forceps. All dissections were performed in Petri dishes with a silicone or beeswax layer.

For adult dissections, SHBs were first pinned dorsally through the pronotum with a minuten pin (Fig. 1a). Fine forceps were then used to break off the elytra by flipping each elytron over 180 degrees (Fig. 1b). The wings are naturally folded and may require a needle probe to unfurl them (Fig. 1c). The wings were clipped with iris scissors, and minuten pins were inserted near the base of each wing (Fig. 1d). The Petri dish was then flooded with distilled water until the specimen was fully submerged. Iris scissors were then used to cut a square in the dorsal surface of the abdomen. This process can be guided by cutting into the membranous surfaces between the abdominal tergites (Fig. 1e). The square piece of cut exoskeleton was then removed with forceps and discarded (Fig. 1f). Organ tissues should be examined separately for the presence of microorganisms. The organs of preserved beetles (previously in 70% ethanol) were examined on slides with lactofuchsin, while the organs of living beetles were examined on slides with Ringer's solution. All tissue inspections were performed with a phase contrast microscope at 400x.

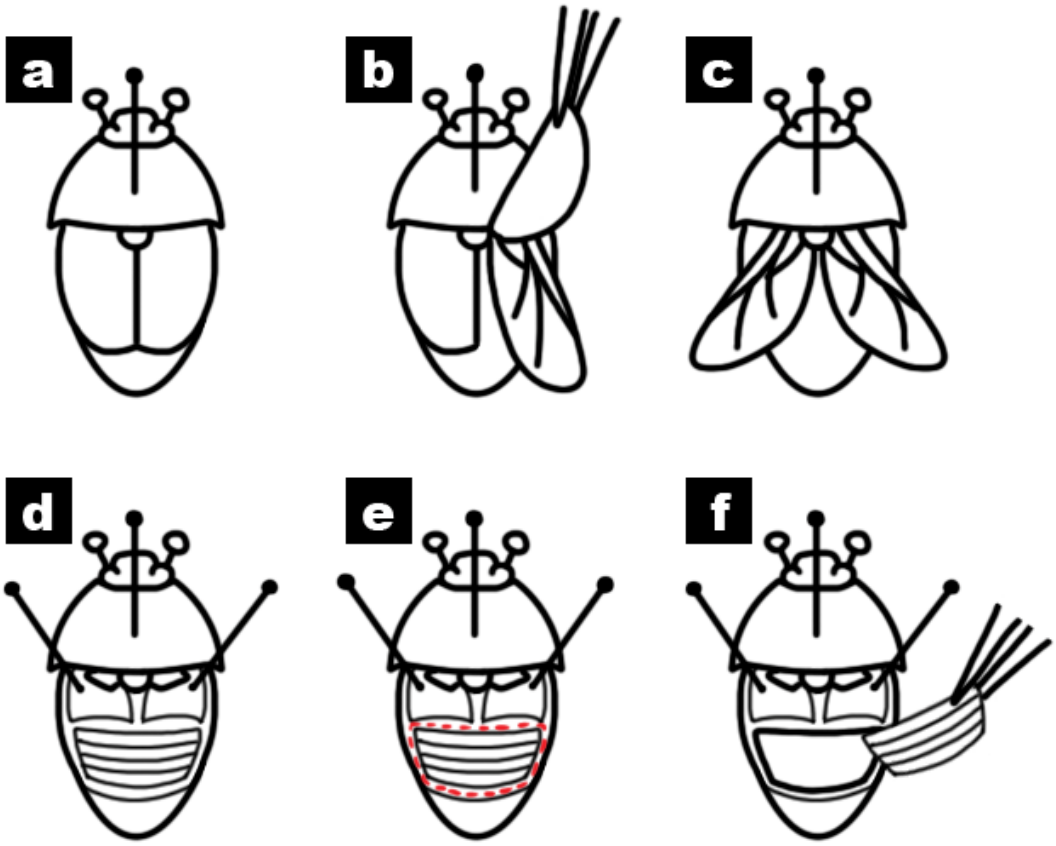


Fig 1. Steps involved in dissecting an adult SHB: **a**, insert minuten pin through pronotum; **b**, remove elytra; **c**, elytra removed with wings unfolded; **d**, clip wings and insert pins near the base of the wings; **e**, make incision around abdominal tergites; **f**, remove section of tergites with forceps to expose internals.

*Larval dissections.*

For larval dissections, SHB larvae were placed dorsal side up and pinned through the head with a minuten (Fig. 2a). A second minuten was inserted on the right side near the posterior end of the larva (Fig. 2a). This second minuten acts as a temporary support and is later removed. Iris scissors were then used to make an incision from the base of the head to the tip of the posterior end (Fig. 2b). Making a perpendicular cut at the base of the head and at the posterior end (overlapping the first incision) will aid in opening the exoskeleton flaps that will be created by the lengthwise incision. The left flap of the larva can be gently brushed open with a minuten. Two minutens were used to hold the left flap of the body open (Fig. 2c). The support minuten on the right side was removed (Fig. 2d) and two more were used to keep the right flap open (Fig. 2e). This allows for a full view of the internal organs. The organs of preserved larvae were stained with lactofuchsin, while the organs of living larvae were examined on slides with Ringer's solution. All tissue inspections were performed with a phase contrast microscope at 400x.

Post-feeding (wandering) larvae were significantly easier to dissect and inspect than feeding stage larvae. Puncturing the midgut of a feeding stage larva will result in pollen (Fig. 3) and other food debris (Fig. 4) filling the body cavity, making inspection for pathogens significantly more difficult. We recommend taking other organ samples first, leaving the midgut sample for last to avoid getting gut contents in the body cavity.

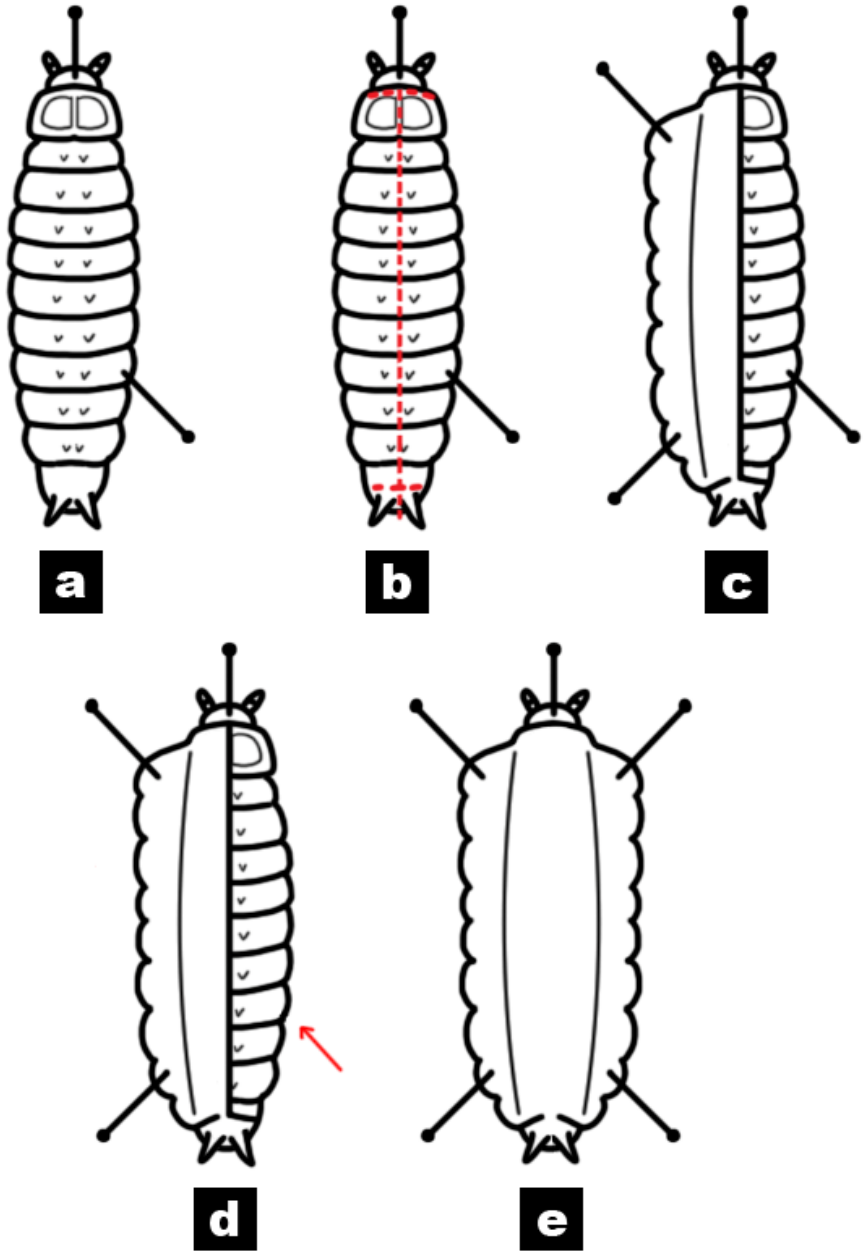


Fig. 2. Steps involved in dissecting a larval SHB: **a**, insert minuten pin near base of head and about 2/3 down the body on the right side; **b**, make incisions near base of head, down the dorsal surface, and at the posterior end; **c**, open left flap using a minuten pin, then pin flap down at anterior and posterior ends; **d**, remove minuten on right side of body; **e**, repeat step c for the right flap to expose the internal organs.

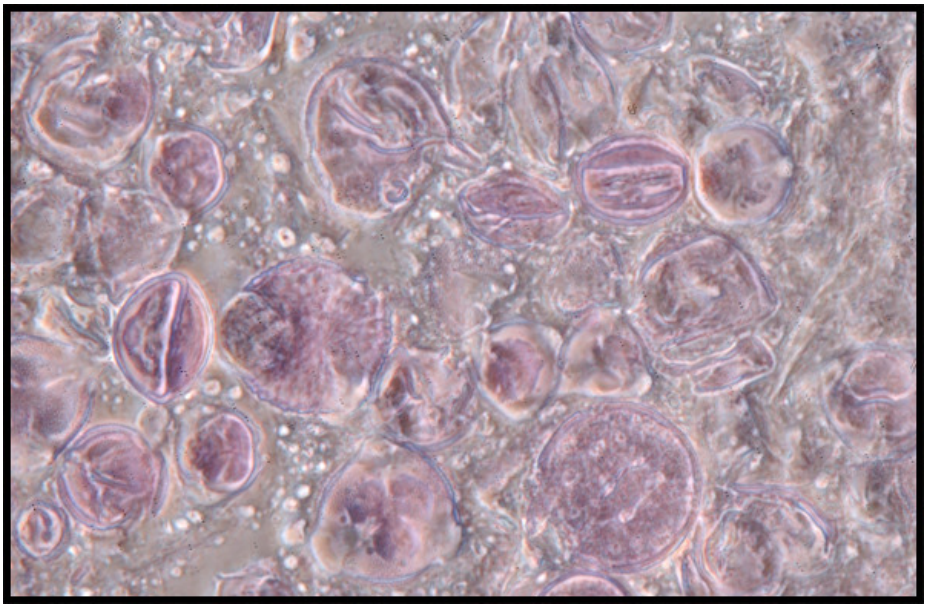

Fig. 3. Pollen was commonly found in the midgut of feeding stage SHB larvae.

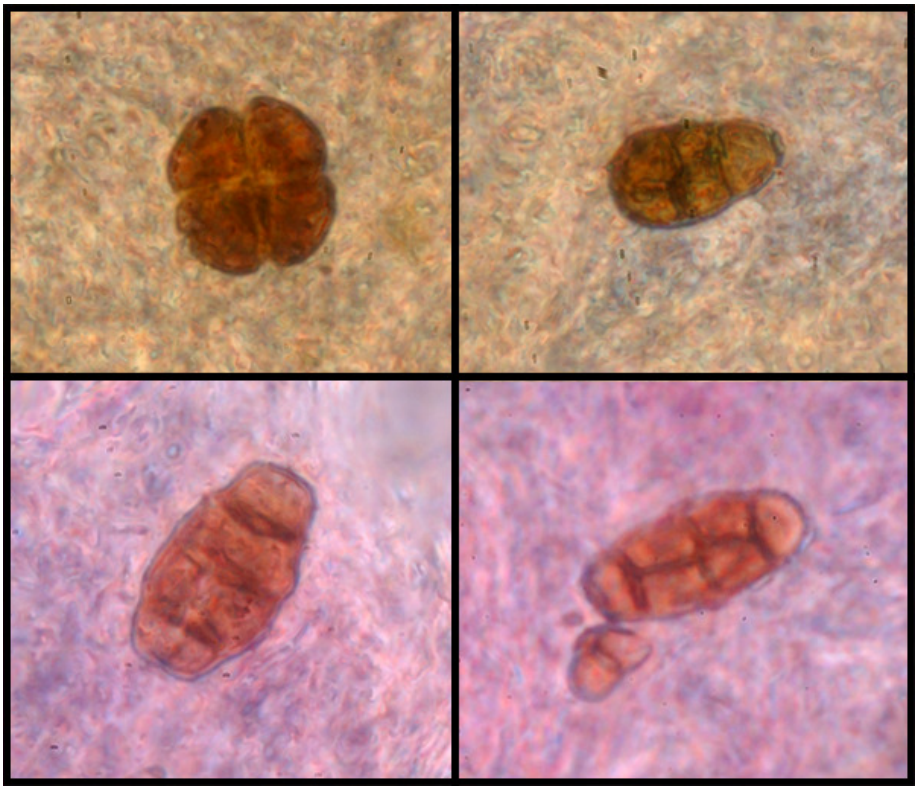

Fig. 4. *Alternaria* fungal spores were fairly common in the midgut of SHB larvae.

**Results and Discussion.**

No dissection techniques have been published on either the adult or larval SHB. Several dozen healthy beetles were used to practice on as the technique was developed. The true difficulty lies in the small size of SHBs. Pinning too close to the edge of a specimen may result in tearing when the organism is gently moved, and pinning too far into the specimen may damage the organs. Preserved adults are prone to breaking in half at the pronotum, so pinning through the pronotum alone is not recommended for support. The dissection of live adults is preferred for observing living pathogens; however, live adults swallow air and usually have air bubbles in the alimentary canal. Chilling beetles in a cold room before dissection in water may reduce the occurrence of air bubbles in the alimentary canal.

These dissection techniques for *A. tumida* adults and larvae make it possible to relatively rapidly dissect this honey bee pest and examine the midgut, fat body, Malpighian tubules, hindgut, and other organs and tissues for microbial pathogens.

**References.**

Cabanillas H.E., Elzen P.J. (2006) Infectivity of entomopathogenic nematodes (Steinernematidae and Heterorhabditidae) against the small hive beetle *Aethina tumida* (Coleoptera: Nitidulidae), J. Apic. Res. 45, 49-50.

Ellis J.D., Rong I.H., Hill M.P., Hepburn H.R., Elzen P.J. (2004) The susceptibility of small hive beetle (*Aethina tumida* Murray) pupae to fungal pathogens, Am. Bee J. 144, 486-488.

Ellis J.D., Spiewok S., Delaplane K.S., Buchholz S., Neumann P., Tedders W.L. (2010) Susceptibility of *Aethina tumida* (Coleoptera: Nitidulidae) larvae and pupae to entomopathogenic nematodes. J. Econ. Entomol. 103(1), 1-9.

Mürrle T.M., Neumann P., Dames J.F., Hepburn H.R., Hill M.P. (2006) Susceptibility of adult *Aethina tumida* (Coleoptera: Nitidulidae) to entomopathogenic fungi, J. Econ. Entomol. 99, 1-6.

## Chapter 4: Atlas of Adult Small Hive Beetle (*Aethina tumida*) External Morphology

### Introduction.

The small hive beetle (Coleoptera: Nitidulidae, SHB) is an invasive pest of honey bee colonies in the United States (Elzen et al. 1999). These beetles can ruin honey by contaminating it with their feces and by vectoring *Kodamaea ohmeri*, a mutualistic yeast that causes fermentation (Schmolke 1974, Benda et al. 2008). In addition to honey damage, SHBs will also consume bee eggs, brood, and pollen if there are not enough patrolling worker bees to keep beetles off the combs (Schmolke 1974, Elzen et al. 1999). These beetles have spread rapidly through migratory beekeeping practices and can now be found in at least 31 states (Neumann and Ellis 2008). Because the SHB is a problem that we cannot avoid, we should focus on learning as much as we can about it.

There is little published information on the external morphology of the SHB. Murray, who first described the species in 1867, provided a detailed description of the adult beetle's shape and form but scarcely touched upon such things as the legs, mandibles, maxillae, and antennae. Murray did not describe the larvae because he only had access to two preserved adult specimens (Murray 1867). Schmolke (1974) described the life cycle and included photographs of the reproductive parts and life stages in his project report from the University of Rhodesia. One of his major contributions was developing a non-lethal method for sexing beetles (Schmolke 1974). Further descriptions of the adult and larval stages were provided by Menier and Jouan (2003). Their article clearly illustrates the antennae, aedeagus, and mature larva (Menier and Jouan 2003). There are brief descriptions made only of the male adult (head, pronotum, scutellum, elytra, legs, abdomen, aedeagus) and mature larvae (Menier and Jouan 2003).

Aside from the three papers cited above, there have been no other works on the morphology of this important pest of honey bees. The objective of our study was to photograph the external morphology of adult male and female small hive beetles and relate their morphology to their life history.

**Materials and Methods.**

*Preparation of specimens for photography.*

A colony of SHB was established in the laboratory. Adult SHBs from this colony were used for photography. Specimens were either killed by freezing or submersion in alcohol for at least an hour. In many cases it was necessary to clean the specimens. This was done by rinsing the specimens in warm water with a small amount of dish washing liquid. A fine soft-bristled brush was used to remove pollen, feces, and other debris from the specimens. The specimens were then rinsed in clean water. Specimens were allowed to dry on a KimWipe™ after cleaning. Adults were gently rubbed with a rolled-up KimWipe™ in order to "fluff up" their setae for a more natural appearance, otherwise the setae were flattened and pressed to the body.

Some photographs were taken of dry specimens while other photographs were taken of specimens submerged in water. All dry specimens were photographed on the lid of a Petri dish. Submerged specimens were photographed in water in a Petri dish with a silicone bottom. Isolated mandibles, legs, and other appendages were submerged in a drop of water to prevent air currents or static electricity from propelling the small pieces off of the Petri dish. Submersion in water also helped to diffuse light.

*Photography equipment and software.*

Photographs were taken with a Nikon D200 digital camera mounted on a Leica Wild M420 stereo microscope. Specimens were evenly lit with a dual Y-shaped fiber optic light. If

light diffusion was necessary to prevent harsh lighting, a piece of rolled up vellum paper, foam drinking cup, rolled up wax paper, or transparent plastic cups were also used to achieve diffuse lighting. These materials were placed around the specimen to diffuse the light.

Approximately 40 serial photographs were taken of each specimen, starting with an in-focus image at the top of the specimen (such as the peak of the elytra) to an in-focus image at the bottom of the specimen (such as the tarsi). Zerene Stacker (Zerene Systems LLC), an image stacking program, was then used to combine the images into a single in-focus image. DMap (depth map) was the preferred stacking method. The combined image was then edited in Adobe Photoshop CS3 with the aid of an Intuos®4 tablet. Edits included color balance, sharpening, removal of the background, and cloning out unwanted particles or debris.

*Labeling*

SHB photographs were labeled using the abbreviations Robert Evans Snodgrass used in is his book, Principles of Insect Morphology (Snodgrass, 1993). When an abbreviation was not available, one was created in a style similar to Snodgrass. Abbreviations are defined in the caption of each figure.

**Results and Discussion.**

*Form and color.*

Both male and female adult SHBs are broadly oval and moderately convex (Fig. 1). The size of adult beetles ranged from about 5.6 to 5.8 mm long and 3.2 mm wide (Ellis et al. 2002a). Mature adults are yellow-brown to dark brown in color. There are no markings or patterns. Setae are golden-brown (Murray 1867). Both dorsal and ventral surfaces are pubescent (Gillogly 1965). Pronounced pubescence occurs on the lateral edges of the pronotum and elytra (Murray 1867).

Females appeared to be morphologically similar to males. Males are generally shorter in length than females (Schmolke 1974, Ellis et al. 2002a), but because variation exists in the lengths of both sexes, size alone should not be used as an indicator of sex (Schmolke 1974).

As their name suggests, SHBs are relatively small, about one-third the size of a honey bee worker. Worker bees will attack these beetles and remove beetle eggs if they are out in the open. The small size of the adult beetles allows them to fit into areas that are inaccessible to worker bees. Laying eggs in cracks and crevices ensures that their eggs will not be removed by vigilant bees.

The dome-like shape of the small hive beetle has a defensive function. There are no protrusions from the exoskeleton. Adult SHBs can retract their antennae, head, and legs to become a compact oval, making it difficult for worker bees to grab the beetle from any angle. Worker bees are rarely able to pry the beetle from its turtle-like defensive posture and often give up after some time (Schmolke 1974). The SHB's dome-like shape also deflects the stings of worker bees and is thick enough to prevent the sting from penetrating (Lundie 1940, Schmolke 1974).

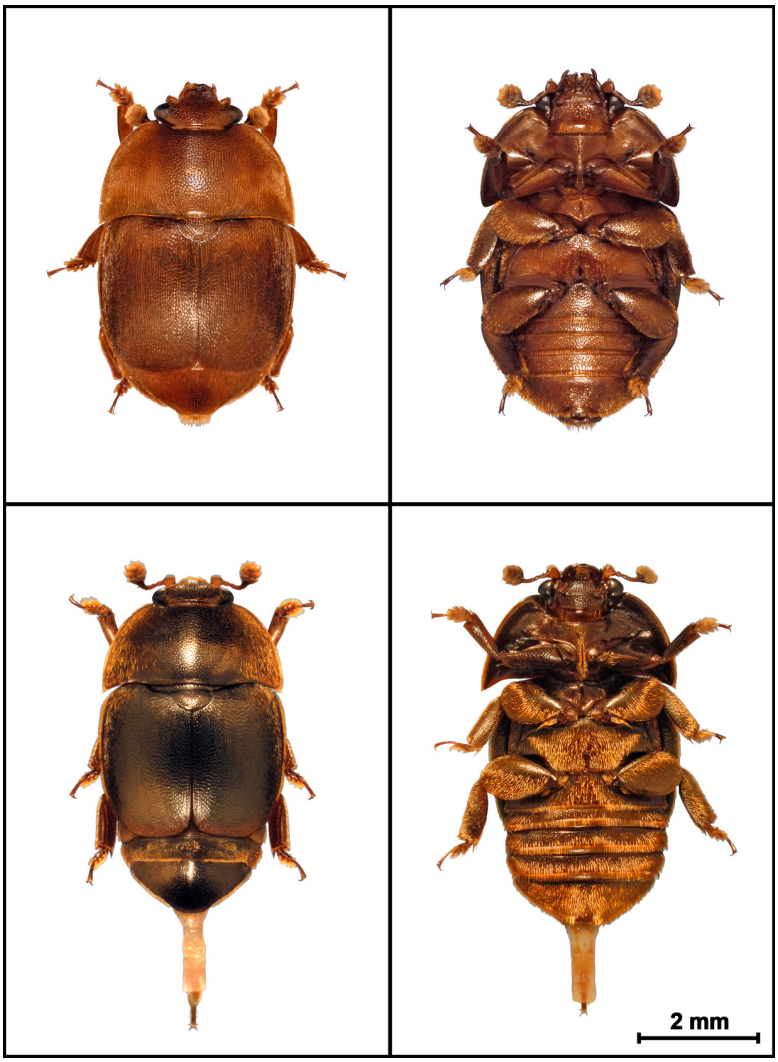


Fig. 1. Adult SHBs. Top, male; bottom, female. Left, dorsal view; right, ventral view.

*Head.*

The head is prognathous, punctate, and moderately flattened dorsoventrally (Fig. 2). The compound eyes are large, laterally-positioned, and protrude from the head (Fig. 2) (Habeck 2002). Setae are present on the compound eyes (Fig. 2) (Habeck 2002). No ocelli are present. The underside of the head has grooves for reception of the antennae (Fig. 2).

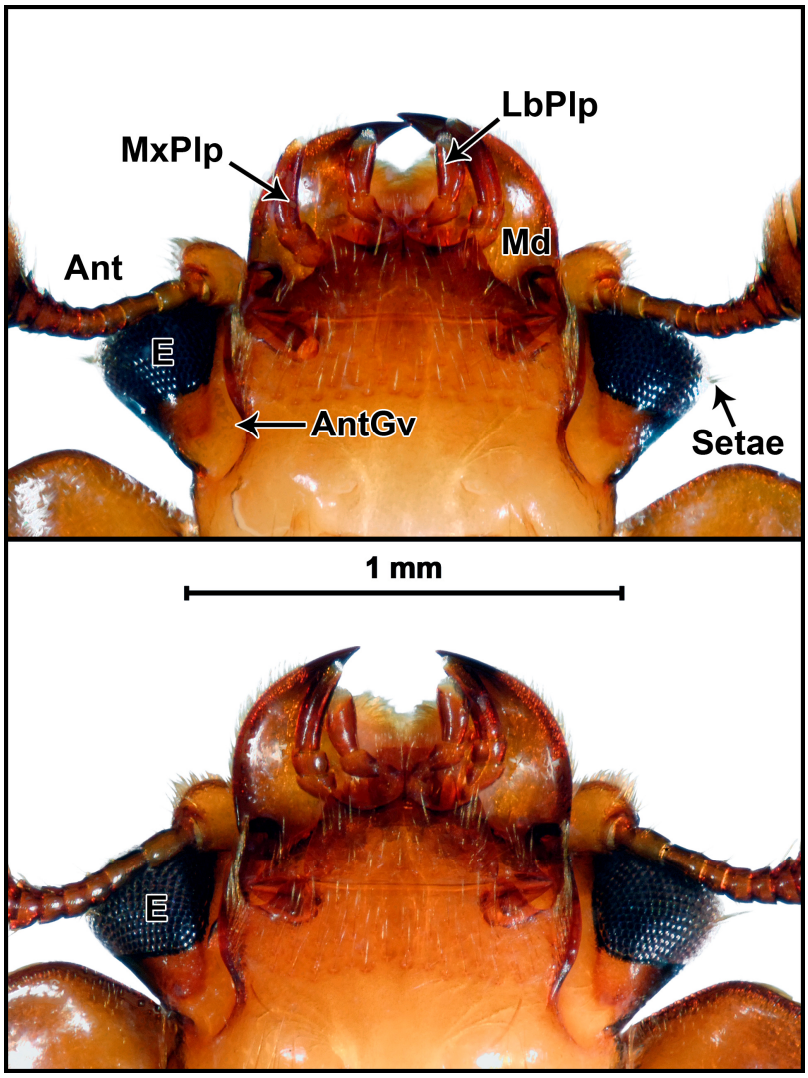


Fig. 2. Ventral view of the SHB head. Top, male; bottom, female. *Ant* - antenna, *AntGv* - antennal groove, *E* - compound eye, *LbPlp* - labial palp, *Md* - mandible, *MxPlp* - maxillary palp, *Setae* points out setae on the compound eyes.

*Antennae.*

The antennae arise from between the protruding compound eyes and the base of the mandibles (Fig. 2) (Habeck 2002). The 11-segmented antennae are capitate with a three-segmented club (Fig. 3). The scape is enlarged with a dense layer of setae along its anterior edge.

The antennae can be withdrawn into grooves on the underside of the head (Fig. 4) (Habeck 2002), preventing them from being grabbed by the mandibles of attacking worker bees. Worker bees that are able to grab on to exposed extremities are likely to tear them off (Schmolke 1974).

Adult SHBs use their sensitive antennae to detect volatiles associated with honey bees and their hive products. Suazo et al. (2003) found that SHBs responded strongly to odors from live worker bees, freshly collected pollen, unripe honey, and slumgum. Torto et al. (2007) found that SHBs are attracted to honey bee alarm pheromone. Remarkably, SHBs are able to detect isopentyl acetate, a component of alarm pheromone, in concentrations lower than even guard and worker bees (Torto et al. 2007). Adult SHBs probably find honey bee colonies by the odors and volatiles given off by the colony, but the distance at which they can detect these volatiles is unknown (Suazo et al. 2003, Torto et al. 2007). It has been stated that adult beetles can detect stressed honey bee colonies from eight to 10 miles away (Wenning 2001), but no studies have been conducted to confirm this assertion.

Little is known about the mating and aggregation behaviors of the SHB. No pheromones have been identified. However, some other nitidulid beetles have aggregation pheromones (Bartelt 1999), so it is possible that small hive beetles may also have aggregation pheromones that have not yet identified. In addition, adult SHBs may use other cues for aggregation such as colony volatiles and fermentation products (Spiewok et al. 2007, Torto et al. 2007).

SHB antennae play a vital role in their behavioral mimicry of bees (Neumann et al. 2001, Ellis et al. 2002b, 2003b). Honey bees are able to imprison beetles within propolis prisons (Neumann et al. 2001, Ellis 2002, Ellis et al. 2003b). A gap is always left in the propolis wall so that guard bees can be stationed to prevent beetles from escaping (Ellis et al. 2003a). Under these

conditions, one might expect that imprisoned SHBs would die from starvation, but they do not. Adult beetles approach guard bees and engage in antennal contact. Sometimes guard bees are apparently fooled into thinking that they are being approached by a hungry bee and regurgitate honey for the SHB (Ellis et al. 2002b). In this way, imprisoned SHBs are able to extend their prison life for weeks or even months, increasing the likelihood that they will eventually escape back into the hive to reproduce (Neumann et al. 2001).

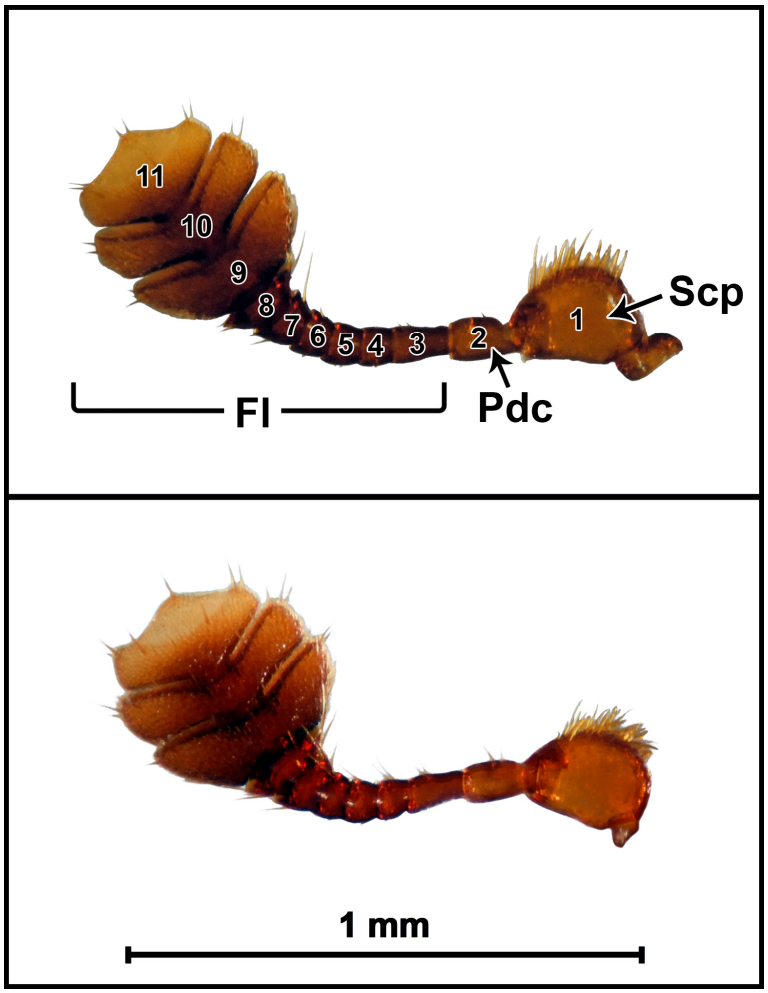


Fig. 3. Ventral view of the SHB antenna. Top, male; bottom, female. *Fl* - flagellum, *Pdc* - pedicel, *Scp* - scape.

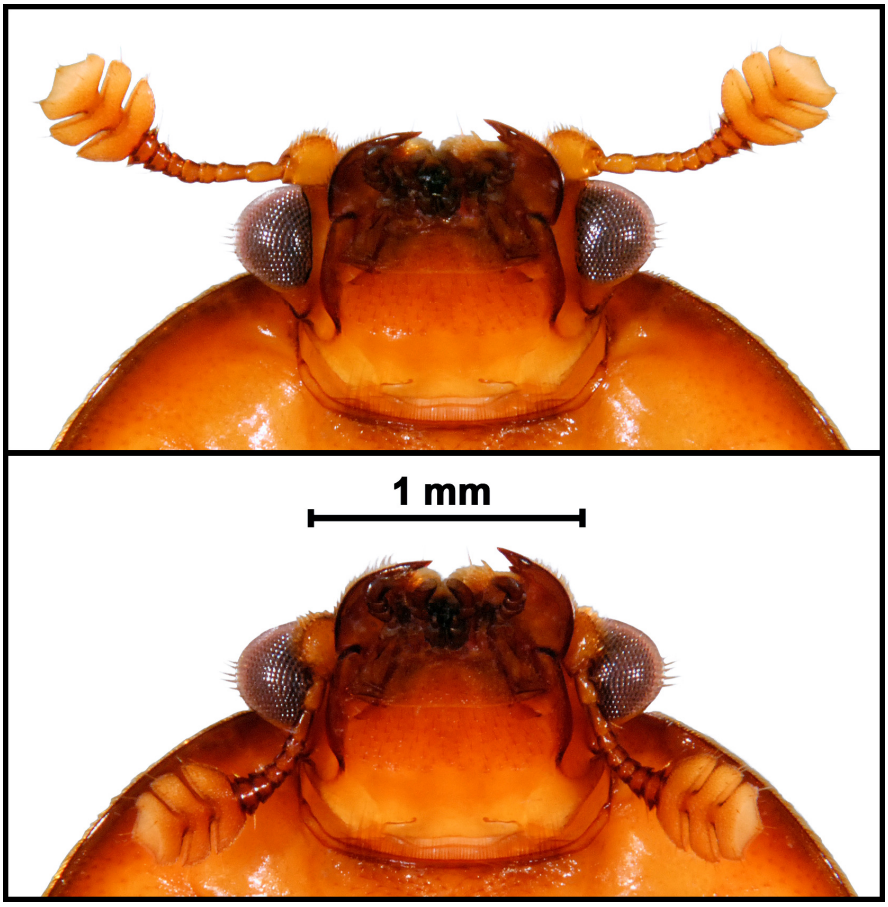


Fig. 4. Ventral view of a female SHB head showing retraction of the antennae into the antennal grooves.

*Mouthparts.*

The mandibles are broad and bidentate with the inner denticle being smaller and shorter than the outer denticle (Fig. 5) (Habeck 2002). A carina is present on the dorsal surface of the mandibles (Fig. 5). Fringes of setae cover the prostheca (Fig. 5) (Naumann et al. 1991). There appears to be no distinct molar region. The incisor region is heavily sclerotized (Fig. 5). Adult SHBs use their mandibles to feed on bee brood and break apart packed pollen. Adult beetles may use their sharp incisors to crack into the exoskeleton of dead honey bees and other dead insects

when other sources of protein are not available. Females use their incisors to poke holes in sealed comb cells so they can insert their ovipositor and lay eggs on bee pupae (Ellis et al. 2004).

The maxillary palps have four palpomeres; the last palpomere is slender and long (Fig. 6) (Habeck 2002). The galea appears to be absent (Habeck 2002). The lacinia is flattened and brush-like (Fig. 6). The labrum is bilobed (Fig. 6) (Habeck 2002). The labial palps are three-segmented (Fig. 6). The brush-like prostheca and lacinia are likely utilized for moist or liquid foods such as honey or macerated bee brood.

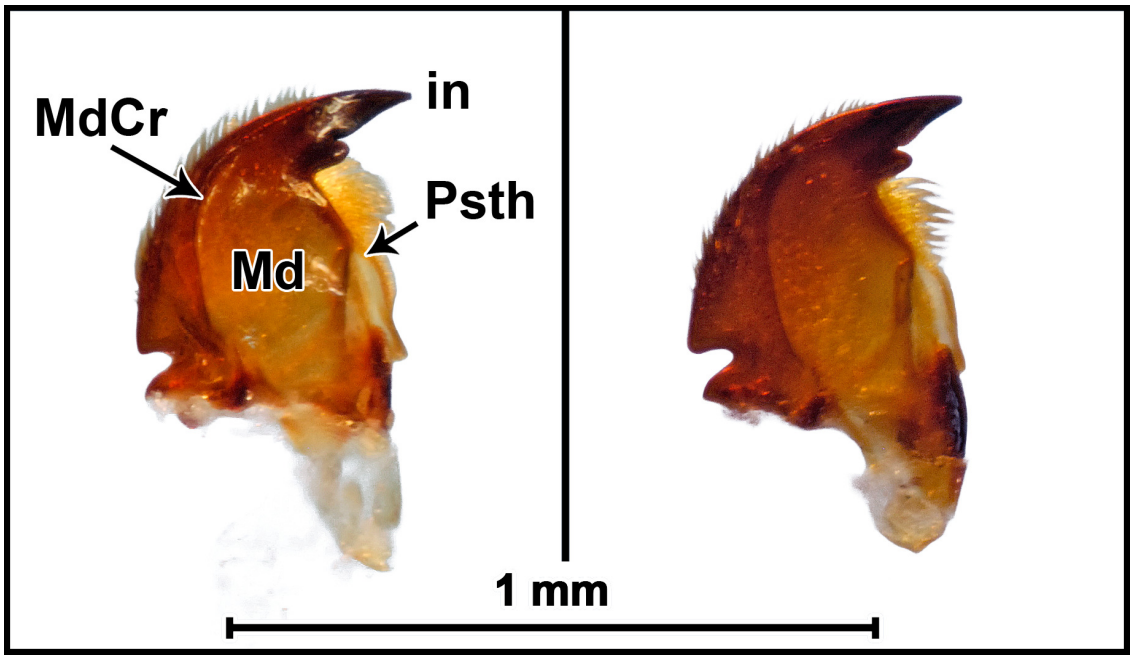


Fig. 5. Dorsal view of the SHB mandible. Left, male; right, female. *in* - incisor region, *Md* - mandible, *MdCr* - mandibular carina, *Psth* - prostheca.

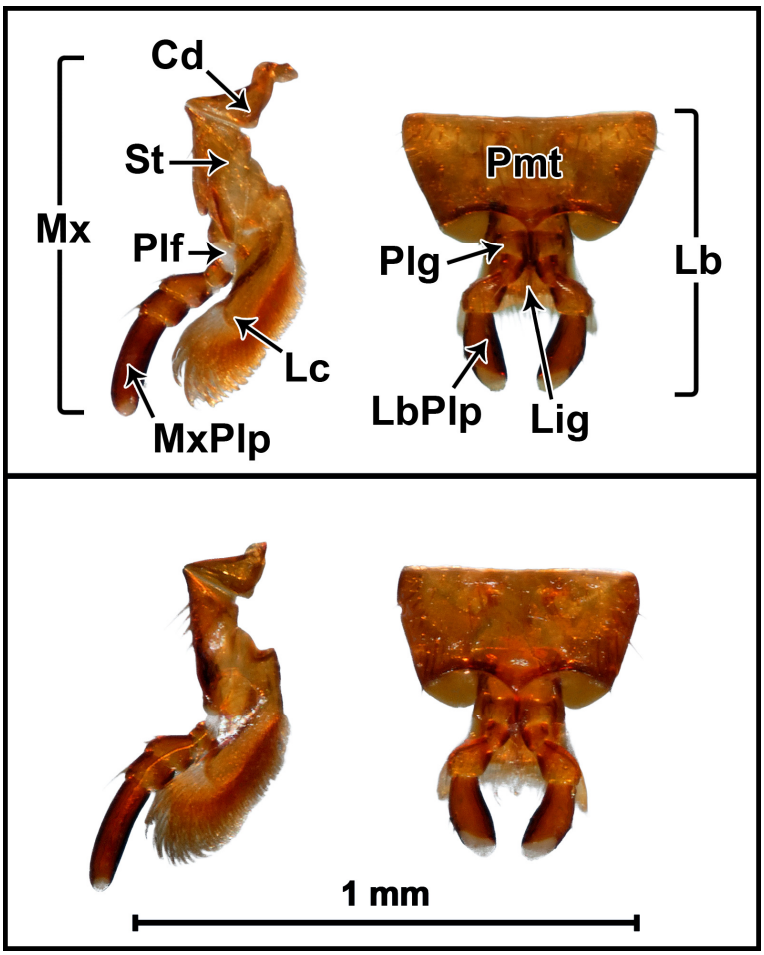


Fig. 6. Ventral view of the SHB maxilla and labrum. Top, male; bottom, female. *Cd* - cardo, *Lb* - labrum, *LbPlp* - labial palp, *Lc* - lacinia, *Lig* - ligula, *Mx* - maxilla, *MxPlp* - maxillary palp, *Plf* - palpifer, *Plg* - palpiger, *Pmt* - postmentum, *St* - stipes.

*Thorax.*

The pronotum is subtrapezoidal. The posterior edge of the pronotum is sinuate (Habeck 2002). The pronotal surface is punctate. The pronotum is widest along the posterior edge. Mesocoxal cavities are closed.

The femora are broad and flattened dorsoventrally (Fig. 7). The femora are grooved, allowing for reception of the tibiae (Fig. 8) (Murray 1867, Habeck 2002). The coxae vary in size

and shape, with the metathoracic coxae being largest (Fig. 7). The trochanters are small and triangular (Fig. 7) (Habeck 2002). The tibiae are short, being no longer than the femora. The tibiae of the meso- and metathoracic legs have spinose, double outer margins (Fig. 7) (Gillogly 1965). There are two tibial spurs present on each leg (Fig. 7).

The legs are retractile and can be hidden beneath the body without significantly raising the body from the surface or substrate. This allows for an adult beetle to hunker down when approached and harassed by worker bees. Unable to grab the beetle, the bees soon give up, allowing the beetle to escape without losing any appendages (Schmolke 1974).

The tarsi have five tarsomeres (Fig. 9). The first three tarsomeres are dilated with setose pads (Fig. 9) (Naumann et al. 1991, Habeck 2002). The fourth tarsomere is greatly reduced in size (Fig. 9). The fifth tarsomere is the longest and bears two simple claws (Fig. 9) (Habeck 2002).

The setose pads may assist males during mating in holding on to the smooth dorsal surface of the females, much in the same way that male diving beetles have suction-cup-like modifications on their foretarsi (Bergsten and Miller 2007). However, this may also not be the case, since both males and females have setose pads on every tarsus.

The elytra are truncate, exposing only the pygidium (Fig. 10). Each elytron is separately rounded. The elytra are almost as long as their combined width. The elytra are punctate and pubescent with longitudinal rows of hairs and punctures (Fig. 11) (Habeck 2002). Together, the elytra form a convex surface. There is a fringe of hairs along the exposed lateral edge (Fig. 11) (Murray 1867, Habeck 2002). The scutellum is semicircular and punctate like the elytra (Fig. 10). The membranous hind wings are long and narrow, being about three times longer than they are wide (Fig. 12). The wings have reduced venation, and the anterior veins are thickened and

amber-colored (Fig. 12). There is an area of heavy pigmentation along the anterior edge, near to the apex (Fig. 12). The wings are lobed basally (Habeck 2002). Habeck (2002) describes the folding pattern of the wings as "extremely complex."

The elytra aid in preventing worker honey bee stings from penetrating the soft, concealed portions of the abdomen. The combined convex shape of the elytra may cause stings to become deflected. The adult beetles are strong and fast fliers, but the distance they can travel is still unknown. The wings are utilized when moving between host colonies. There have also been reports of adult SHBs following honey bee swarms (Ellis 2004). If SHBs follow swarms, they do so by tracking the swarm by odor with their antennae and flying to keep up with the cluster.

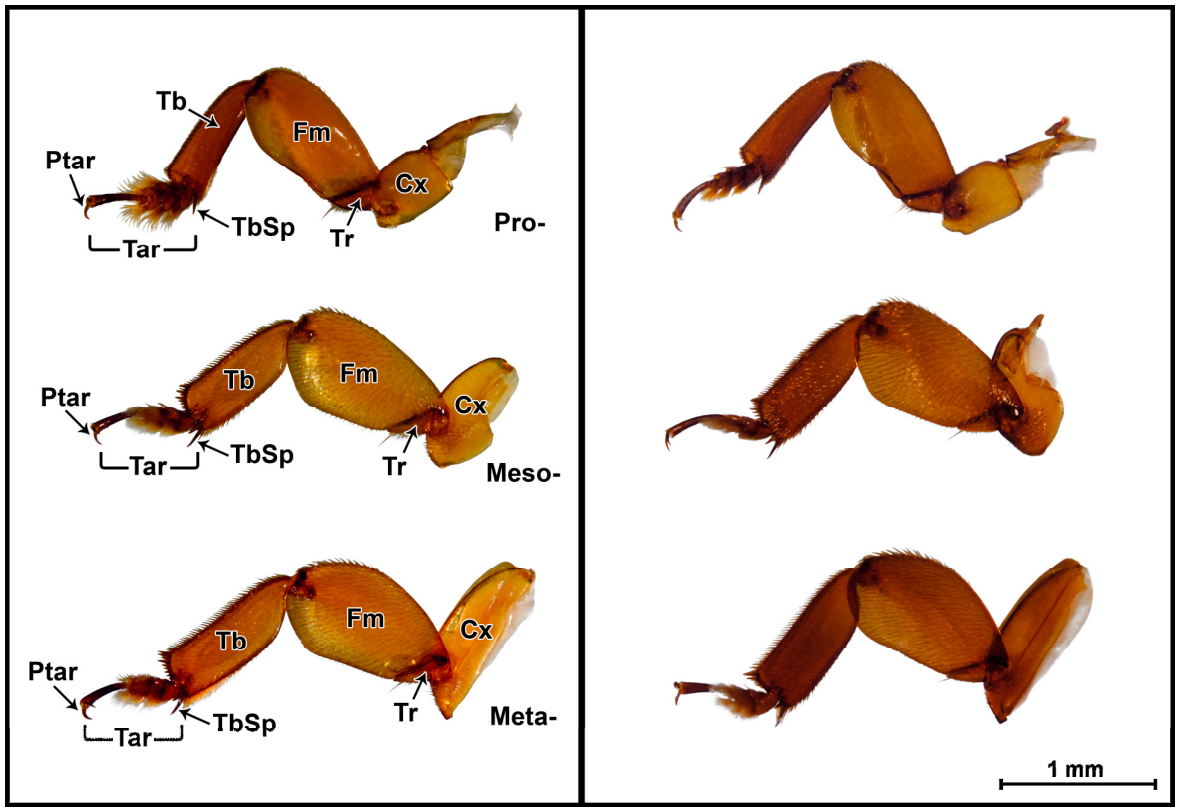


Fig. 7. Ventral view of the SHB legs. Left, male; right, female. *Cx* - coxa, *Fm* - femur, *Ptar* - pretarsus, *Tar* - tarsus, *Tb* - tibia, *TbSp* - tibial spur, *Tr* - trochanter.


Fig. 8. Dorsal view of the metathoracic legs of an adult male SHB showing the retraction of the tibia into the grooved femur.

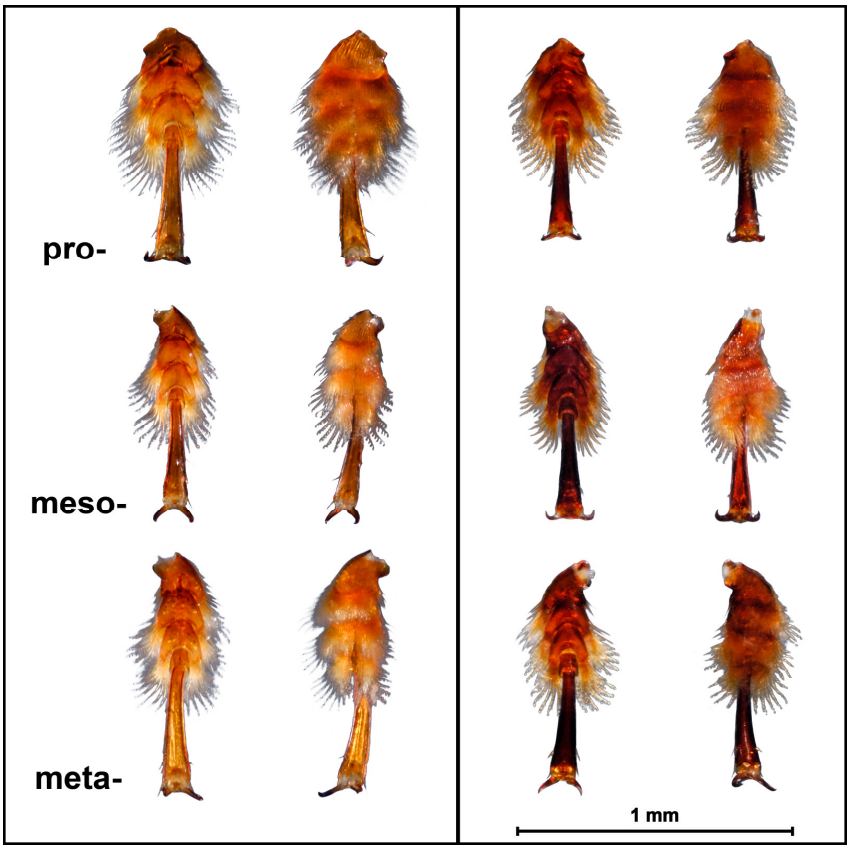


Fig. 9. SHB tarsi shown dorsal first, ventral second. Left, male; right, female.

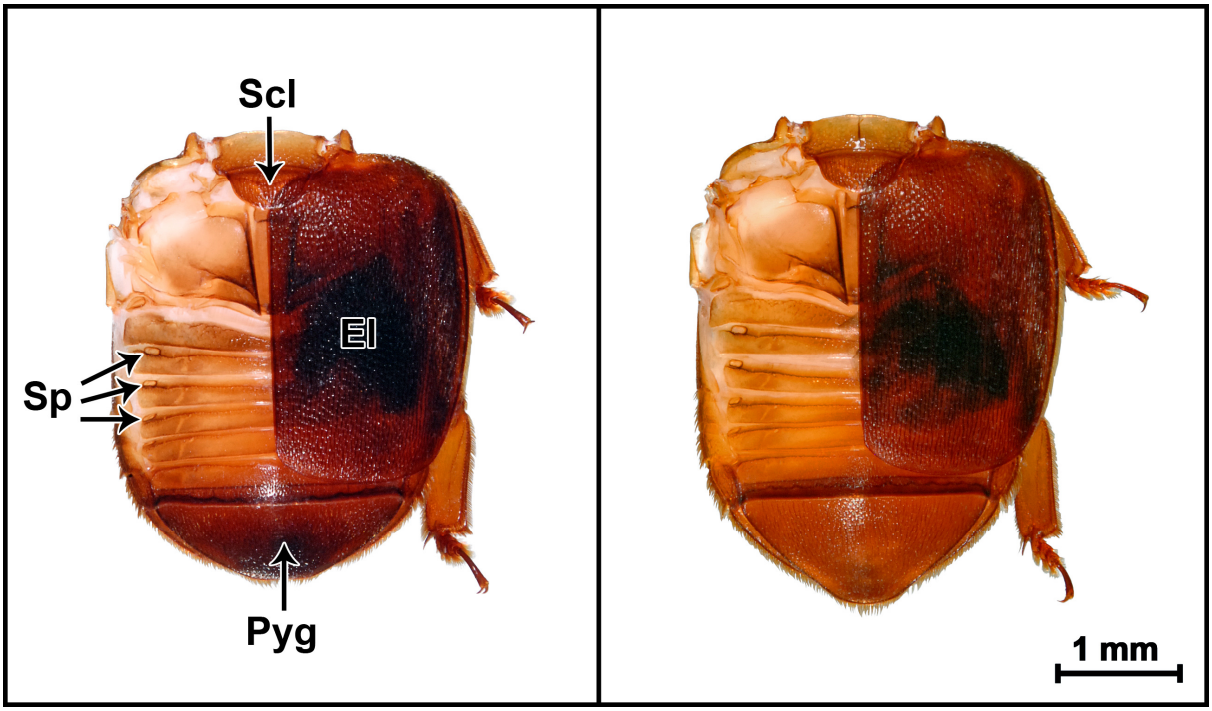


Fig. 10. Dorsal view of the SHB without head and prothorax. Elytron removed from left side. Left, male; right, female. *El* - elytron, *Pyg* - pygidium, *Scl* - scutellum, *Sp* - spiracle.

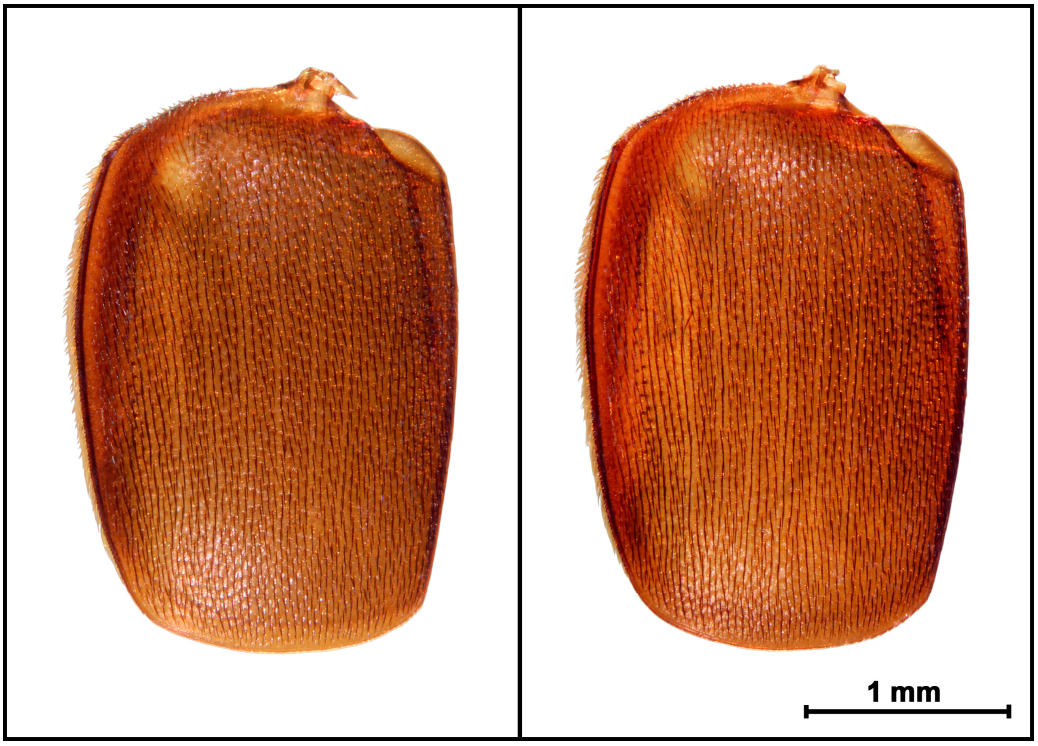


Fig. 11. Dorsal view of the SHB elytron. Left, male; right, female.


Fig. 12. Dorsal view of the SHB wing. Top, male; bottom, female.

*Abdomen.*

There are five visible sternites (=ventrite, Fig. 13) (Habeck 2002). The second, third, and fourth sternites are nearly equal in size. The fifth sternite is the largest. There are seven tergites (Fig. 14). The seventh tergite (=pygidium) is the apparent terminal segment and is the only tergite that completely extends beyond the elytra (Fig. 10) (Gordh and Headrick 2001). The preceding segment (=propygidium) is only partially covered by the elytra (Fig. 10) (Naumann et al. 1991).

The exposed pygidium and propygidium are more sclerotized than the tergites concealed by the elytra (Fig. 14). The pygidium and propygidium serve as a protective extension of the short elytra, deflecting stings and also preventing stings from penetrating.

Schmolke (1974) found that he could determine the sexes of beetles by squeezing them gently around their midsection with a pair of rounded forceps. Beetles can also be sexed without forceps by squeezing them gently between your index finger and thumb. I found it easier to be gentle with them and sex them this way using only my fingers. When squeezed, male SHBs protrude their eighth tergite (Fig. 15). When isolated, the eighth tergite is crescent-shaped (Fig. 16). There is a fringe of setae along the outer margin. Females protrude their ovipositor when squeezed (Fig. 15). The ovipositor is cream-colored with brown stylets (Fig. 15). It can be quite long compared to the female, about one-fourth of her body length when fully extended (Fig. 1).

The slender flexible ovipositor is adapted for laying eggs in narrow spaces. Worker bees are efficient at removing unprotected beetle eggs, so eggs must be laid in hard-to-reach places to ensure that they hatch (Schmolke 1974). In a colony with many patrolling worker bees, female beetles cannot lay their eggs directly on protein sources and must lay their eggs in cracks at the periphery of the hive (Lundie 1940, Schmolke 1974). In weak colonies with fewer patrolling bees, female beetles may chew holes in sealed comb cells and insert their ovipositor to lay eggs on bee brood (Ellis et al. 2004). This is risky oviposition behavior in a strong colony, since the female beetle is vulnerable while out on the comb (Ellis et al. 2004, Ellis and Delaplane 2008).

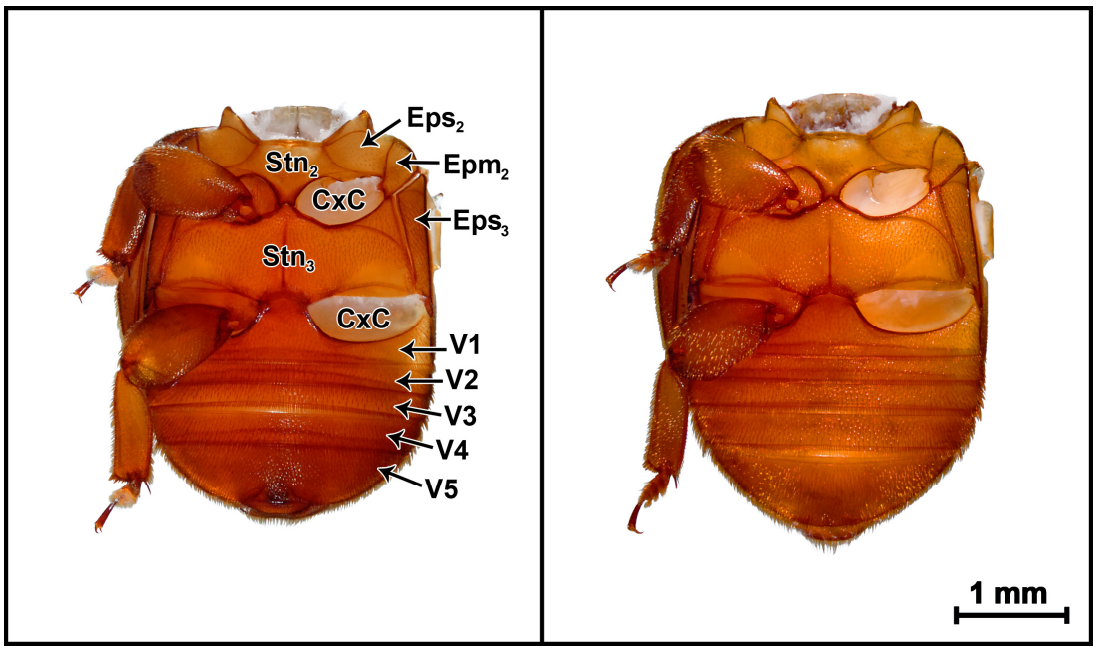


Fig. 13. Ventral view of the SHB without prothorax and head. Legs were removed from the right side. Left, male; right, female. *CxC* - coxal cavity, *Epm*$_2$ - mesepimeron, *Eps*$_2$ - mesepisternum, *Eps*$_3$ - metaepisternum, *Stn*$_2$ - mesosternum, *Stn*$_3$ - metasternum, *V* - ventrite.

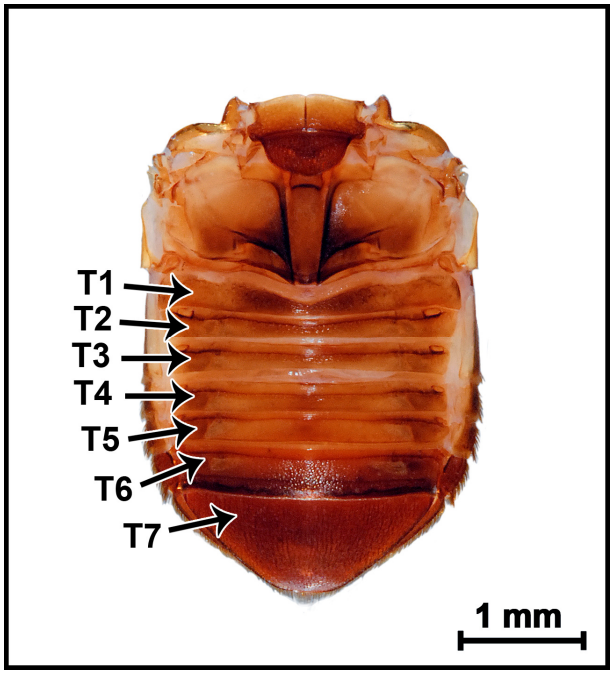


Fig. 14. Dorsal view of the male SHB with wings, elytra, prothorax, and head removed. *T* - tergite. *T6* = propygidium, *T7* = pygidium.

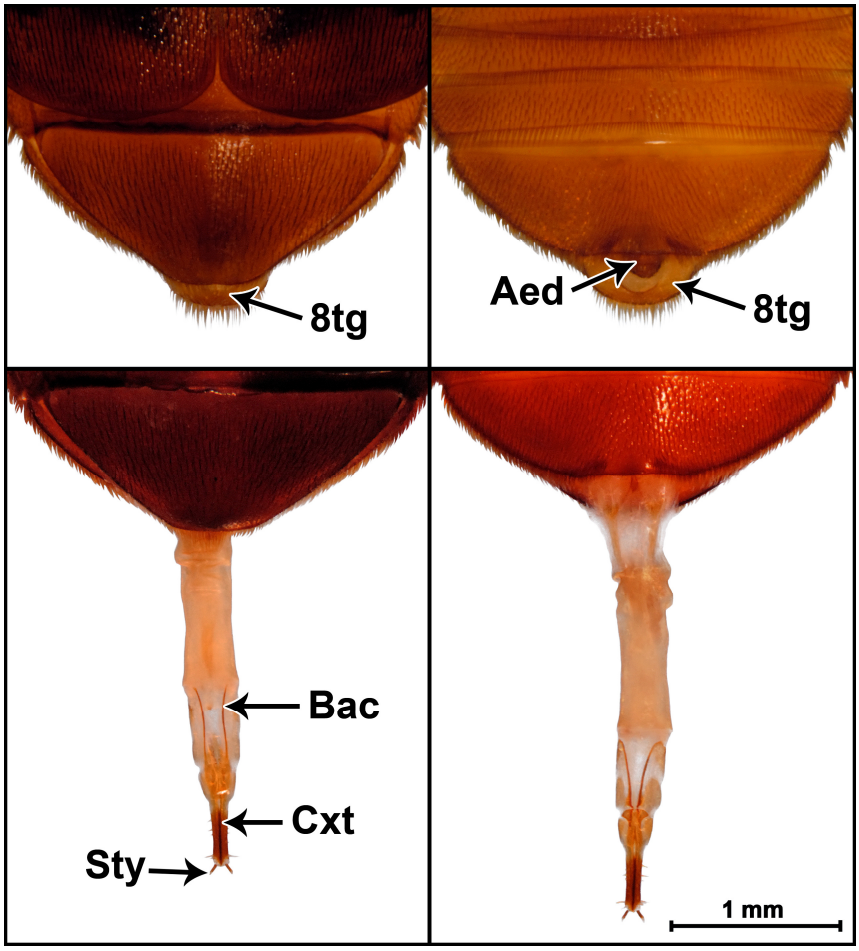


Fig. 15. External view of the reproductive organs of the SHB. Top, male; bottom, female. Left, dorsal view; right, ventral view. *8tg* - 8th tergite, *Aed* - aedeagus, *Bac* - baculus, *Cxt* - coxite, *Sty* - stylet.

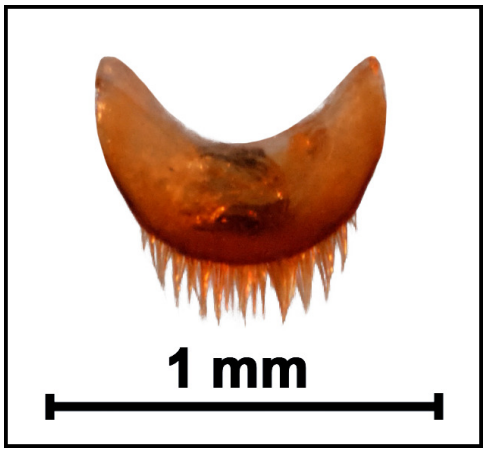


Fig. 16. Dorsal view of an isolated 8th tergite from a male SHB.

**Acknowledgements.**

I would like to thank Dr. Michael Thomas for teaching me how to prepare specimens for photography. In addition, Dr. Thomas also taught me how to use image stacking software and how to clean up photographs with image editing software.

**References.**

Bartelt, R. J. 1999. Pheromones of non-lepidopteran insects associated with agricultural plants, CABI publishing, Wallingford, pp. 69-89.

Benda, N. D., D. Boucias, B. Torto, and P. Teal. 2008. Detection and characterization of *Kodamaea* ohmeri associated with small hive beetle *Aethina tumida* infesting honey bee hives. J. Apic. Res. and Bee World 47(3): 194-201.

Bergsten, J., and K. B. Miller. 2007. Phylogeny of diving beetles reveals a coevolutionary arms race between the sexes. PLoS ONE 2(6): e522. doi:10.1371/journal.pone.0000522.

Ellis, J. D. 2002. Life behind bars: why honey bees feed small hive beetles. Am. Bee J. 142(4): 267-269.

Ellis, J. D., K. S. Delaplane, and W. M. Hood. 2002a. Small hive beetle (*Aethina tumida* Murray) weight, gross biometry, and sex proportion at three locations in the southeastern United States. Am. Bee J. 142(7): 520-522.

Ellis, J. D., C. W. W. Pirk, H. R. Hepburn, G. Kastberger, and P. J. Elzen. 2002b. Small hive beetles survive in honeybee prisons by behavioral mimicry. Naturwissenschaften 89: 326-328.

Ellis, J.D., R. Hepburn, K. S. Delaplane, P. Neumann, and P. J. Elzen. 2003a. The effects of adult small hive beetles, *Aethina tumida* (Coleoptera: Nitidulidae), on nests and flight activity of Cape and European honey bees (*Apis mellifera*). Apidologie 34: 399-408.

Ellis, J. D., H. R. Hepburn, A. M. Ellis, and P. J. Elzen. 2003b. Social encapsulation of the small hive beetle (*Aethina tumida* Murray) by European honeybees (*Apis mellifera* L.). Insectes Soc. 50: 286-291.

Ellis, J. D. 2004. The ecology and control of small hive beetles (*Aethina tumida* Murray). Ph.D. dissertation, Rhodes University, Grahamstown, South Africa.

Ellis, J. D., K. S. Delaplane, C. S. Richards, R. Hepburn, J. A. Berry, and P. J. Elzen. 2004. Hygienic behavior of Cape and European *Apis mellifera* (Hymenoptera: Apidae) toward *Aethina tumida* (Coleoptera: Nitidulidae) eggs oviposited in sealed bee brood. Ann. Entomol. Soc. Am. 97(4): 860-864.

Ellis, J. D., and K. S. Delaplane. 2008. Small hive beetle (*Aethina tumida*) oviposition behavior in sealed brood cells with notes on the removal of the cell contents by European honey bees (*Apis mellifera*). J. Apic. Res. and Bee World 47(3): 210-215.

Elzen, P. J., J. R. Baxter, D. Westervelt, C. Randall, K. S. Delaplane, L. Cutts, and W. T. Wilson. 1999. Field control and biology studies of a new pest species, *Aethina tumida* Murray (Coleoptera, Nitidulidae), attacking European honey bees in the Western Hemisphere. Apidologie 30: 361-366.

Gillogly, L. R. 1965. A key to the genera of the subfamily Nitidulinae (Nitidulidae, Coleoptera) and description of a new genus and a new species. Occasional papers of the Bureau of Entomology, California Department of Agriculture. 8: 1-24.

Gordh, G., and D. H. Headrick. 2001. A dictionary of entomology. CABI Publishing, New York, New York.

Habeck, D. H. 2002. Nitidulidae Latreille 1802, pp. 311-315. *In* R. H. Arnett, M. C. Thomas, P. E. Skelley, and J. H. Frank (eds.), American Beetles, Polyphaga: Scarabaeoidea through Curculionoidea, vol. 2. CRC Press LLC, Boca Raton, FL.

Lundie, A. E. 1940. The small hive beetle, *Aethina tumida*. Sci. Bull. 220, Union of South Africa, Dept Agric Forestry, 30p.

Menier, J., and M. Jouan. 2003. Biologie et description d'*Aethina tumida* Murray, 1867, espéce invasive déprédatrice de l'Abeille mellifére (Coleoptera, Nitidulidae). Bulletin de la Société entomologique de France. 108(3): 323-328.

Murray, A. 1867. List of Coleoptera received from Old Calabar, on the west coast of Africa. The Annals and Magazine of Natural History, London 19: 176-177.

Naumann, I. D., P.B. Carne, E. S. Lawrence, J. P. Nielsen, R. W. Spradbery, R. W. Taylor, M. J. Whitten, and M. J. Littlejohn (eds.). 1991. The insects of Australia: A textbook for students and research workers, 2nd ed. Division of Entomology, CSIRO, Australia. Melbourne University Press, Melbourne, Australia, and Cornell University Press, Ithaca, New York.

Neumann, P., C. W. W. Pirk, H. R. Hepburn, A. J. Solbrig, F. L. W. Ratnieks, P. J. Elzen, and J. R. Baxter. 2001. Social encapsulation of beetle parasites by Cape honeybee colonies (*Apis mellifera capensis* Esch.). Naturwissenschaften 88: 214-216.

Neumann, P., and J. D. Ellis. 2008. The small hive beetle (*Aethina tumida* Murray, Coleoptera: Nitidulidae): distribution, biology and control of an invasive species. J. Apic Res. and Bee World 47(3): 181-183.

Schmolke, M. D. 1974. A study of *Aethina tumida*: the small Hive Beetle, Project Report, University of Rhodesia, p. 178.

Snodgrass, R. E. 1993. Principles of insect morphology. Cornell University Press, Ithaca, New York.

Spiewok, S., J. S. Pettis, M. Duncan, R. Spooner-Hart, D. Westervelt, and P. Neumann. 2007. Small hive beetle, *Aethina tumida*, populations I: Infestation levels of honeybee colonies, apiaries and regions. Apidologie 38: 595-605.

Suazo, A., B. Torto, P. E. A. Teal, and J. H. Tumlinson. 2003. Response of the small hive beetle (*Aethina tumida*) to honey bee (*Apis mellifera*) and beehive-produced volatiles. Apidologie 34: 525-533.

Torto, B., D. G. Boucias, R. T. Arbogast, J. H. Tumlinson, and P. E. A. Teal. 2007. Multitrophic interaction facilitates parasite-host relationship between an invasive beetle and the honey bee. Proc. Nat. Acad. Sci. USA 104(20): 8374-8378.

Wenning, C. J. 2001. Spread and threat of the small hive beetle. Am. Bee J. 141(9): 640-643.

## Chapter 5: A Scientific Note on a Protozoan Pathogen of the Small Hive Beetle

The small hive beetle (SHB), *Aethina tumida* (Coleoptera: Nitidulidae), is an invasive pest of European honey bee colonies. Chemical and cultural controls are typically used to keep SHBs at a tolerable level. Little work has been done on the biological control of SHBs. While some generalist fungal pathogens and commercially available nematodes have been reported to attack SHBs (Ellis et al., 2004; Mürrle and Neumann, 2004; Cabanillas and Elzen, 2006; Ellis et al., 2010), and a yeast, *Kodamaea ohmeri,* forms a mutualistic relationship with SHBs (Torto et al., 2007), to date, there have been no published reports of any host-specific predators, parasitoids, or pathogenic protozoa, fungi, nematodes, bacteria, or viruses. We conducted a study to determine if there are any naturally occurring pathogens of SHB adults and larvae in Arkansas and adjacent states. This is the first report of an obligate internal pathogen of SHBs.

Beekeepers were solicited to submit SHB samples at beekeeper meetings. SHBs were sent to us in 70% ethanol by beekeepers and bee removers. In some cases, live adults were collected from apiaries and maintained on honey before being dissected. In 2011, we dissected 749 adults and 230 larvae from 13 counties in Arkansas and one county each in Oklahoma and Missouri (Table 1). The midgut, Malpighian tubules, and fat body from each SHB were examined under a phase contrast microscope at 400x for the presence of microorganisms (Undeen and Vávra, 1997). Lactofuchsin was used for mounting the tissues of preserved specimens, and Ringer's solution was used for fresh specimens (Becnel, 1997).

We observed no microbial pathogens in SHB larvae (n=230). However, we did find a protozoan pathogen in adult SHBs from three Arkansas counties: Crittenden, Pulaski, and St. Francis. Forty adults (5.3%) were found to be infected in preserved and living specimens (n=749). Most of these infected beetles were from a single apiary in St. Francis County, AR

(n=201, 18.4% infected). Of the 189 living adult SHBs collected from apiaries across Arkansas, three lightly infected beetles were found (less than 50 cysts counted per SHB).

Protozoan cysts were found only in the Malpighian tubules of infected adult SHBs. The severity of infections varied among SHB specimens. Malpighian tubules of uninfected or lightly infected beetles were normal in appearance (Fig. 1a) with zero or relatively few cysts. However, Malpighian tubules of heavily infected beetles were greatly swollen, containing thousands of cysts (Fig. 1b). The cysts were lemon-shaped (Fig. 1c) and measured 9.9 (± 0.13) µm in length and 7.1 (±0.05) µm in width (n=65). It seems likely that heavily infected Malpighian tubules had impaired function in life.

We observed no early developmental protozoan life stages (such as primary or secondary trophozoites in the Amoebida or sporozoites and merozoites in the Neogregarinida) in preserved or living SHB specimens. Precise identification of this protozoan morphologically, based only on cysts, was not possible. Identification of the cysts would be very difficult even with molecular techniques. Currently, there is no effective method for isolating the cysts from host tissue and DNA. Obtaining nucleic acids and rRNA gene sequences from cysts is extremely difficult and expensive (J. Silberman, unpubl. data). Therefore, we were unable to identify this protozoan further at this time.

Based on the organ specificity (only the Malpighian tubules were infected) and the size and shape of the cysts, this newly discovered protozoan pathogen of SHB could be a species in the order Amoebida, perhaps in one of the three genera reported as pathogens of insects: *Malameba*, *Malphigamoeba*, or *Malpighiella* (Undeen and Vávra, 1997). However, the cysts also resembled those of protozoa in the order Neogregarinida. We consulted two prominent insect pathologists. They stated that the cysts appeared to be neogregarines but they could not be

certain without seeing the immature stages (H. Kaya and J. Lord, unpubl. data). Even though we lacked early stages, it is evident that this protozoan is an obligate internal pathogen. While there are some commensal or weakly pathogenic protozoa found in insects (such as the eugregarines), neogregarines and entomogenous amoebae found in the Malpighian tubules are considered to be obligate pathogens (Tanada and Kaya, 1993).

Future research should include bioassays with this protozoan and SHBs to examine as many life stages of this protozoan as possible, allowing for more precise identification.

**Acknowledgements.**

We thank the California State Beekeeper Association for funding. We would also like to thank the beekeepers, bee removers, and Jon Zawislak for supplying SHBs. Drs. Jeff Silberman, Harry Kaya, and Jeff Lord examined our specimens and provided advice.

**References.**

Becnel J.J. (1997) Complementary techniques: Preparations of entomopathogens and diseased specimens for more detailed study using microscopy, in: Lacey L.A. (Ed.), Manual of techniques in insect pathology, Elsevier, Amsterdam, Netherlands, pp. 337-353.

Cabanillas H.E., Elzen P.J. (2006) Infectivity of entomopathogenic nematodes (Steinernematidae and Heterorhabditidae) against the small hive beetle *Aethina tumida* (Coleoptera: Nitidulidae), J. Apic. Res. 45, 49-50.

Ellis J.D., Rong I.H., Hill M.P., Hepburn H.R., Elzen P.J. (2004) The susceptibility of small hive beetle (*Aethina tumida* Murray) pupae to fungal pathogens, Am. Bee J. 144, 486-488.

Ellis J.D., Spiewok S., Delaplane K.S., Buchholz S., Neumann P., Tedders W.L. (2010) Susceptibility of *Aethina tumida* (Coleoptera: Nitidulidae) larvae and pupae to entomopathogenic nematodes. J. Econ. Entomol. 103(1), 1-9.

Mürrle T.M., Neumann P., Dames J.F., Hepburn H.R., Hill M.P. (2006) Susceptibility of adult *Aethina tumida* (Coleoptera: Nitidulidae) to entomopathogenic fungi, J. Econ. Entomol. 99, 1-6.

Tanada Y., Kaya H.K. (1993) Insect Pathology, Academic Press, San Diego, California.

Torto B., Boucias D.G., Arbogast R.T., Tumlinson J.H., Teal P.E.A. (2007) Multitrophic interaction facilitates parasite-host relationship between an invasive beetle and the honey bee. Proc. Nat. Acad. Sci. USA 104(20), 8374-8378.

Undeen A.H., Vávra J. (1997) Research methods for entomopathogenic Protozoa, in: Lacey L.A. (Ed.), Manual of techniques in insect pathology, Elsevier, Amsterdam, Netherlands, pp. 117-151.

| County and State | SHB Larvae | | SHB Adults | |
|---|---|---|---|---|
| | # dissected | # infected | # dissected | # infected |
| Arkansas Co. AR | 5 | 0 | 12 | 0 |
| Ashley Co. AR | - | 0 | 16 | 0 |
| Boone Co. AR | - | 0 | 9 | 0 |
| Carroll Co. AR | - | 0 | 5 | 0 |
| Cass Co. MO | 38 | 0 | 21 | 0 |
| Craighead Co. AR | - | 0 | 51 | 0 |
| Crittenden Co. AR | - | 0 | 50 | 1 |
| Cross Co. AR | - | 0 | 33 | 0 |
| Drew Co. AR | - | 0 | 11 | 0 |
| Faulkner Co. AR | - | 0 | 25 | 0 |
| Ottawa Co. OK | - | 0 | 21 | 0 |
| Pulaski Co. AR | 35 | 0 | 102 | 2 |
| Saline Co. AR | 38 | 0 | 78 | 0 |
| St. Francis Co. AR | 64 | 0 | 201 | 37 |
| Washington Co. AR | 50 | 0 | 114 | 0 |
| Total | 230 | 0 | 749 | 40 |

Table 1. Number of infected and uninfected SHB larvae and adults in 2011.


Fig. 1a. Healthy Malpighian tubule from an adult SHB (prepared in Ringer's solution).

Fig 1b. Heavily infected, swollen, Malpighian tubule from an adult SHB containing many protozoan cysts (prepared in lacto-fuchsin). Arrows point out some protozoan cysts.

Fig 1c. Close up of a single protozoan cyst.

## Chapter 6: Soil Pathogens of the Small Hive Beetle (*Aethina tumida*) in Arkansas

### Introduction.

The small hive beetle *Aethina tumida* (Coleoptera: Nitidulidae, SHB) is only a minor pest of honey bees in its native range in Africa, but it has been shown to cause considerable damage to honey bees in the United States and Australia (Schmolke 1974, Elzen et al. 2000, Hood 2004). Adults and larvae can cause honey to ferment, and fermented honey is rejected by the bees and should not be sold for human consumption (Hood 2004). The current control measures for SHBs are chemical or cultural. Very little is known about the natural enemies of the SHB and so not much research has been done in the area of biological control.

Schmolke (1974) never found any parasites or disease-causing pathogens in wild caught or laboratory raised beetles. Torto et al. (2010) observed that *Pheidole megacephala*, a generalist ant, preyed upon beetle pupae that were close to the soil surface. It is unlikely that this generalist predator provides significant control since these ants prefer other types of foods more than insect prey (Torto et al. 2010). The entomopathogenic fungi *Metarhizium anisopliae* and *Beauveria bassiana* have been shown to cause pupal mortality (Mürrle et al. 2006). *Aspergillus flavus* and *A. niger* have also been tested, but their toxicity to other animals makes these fungi unsuitable as biological control agents (Ellis et al. 2004a, Richards et al. 2005). The entomopathogenic nematodes *Heterorhabditis bacteriophora* Oswego strain, *H. indica*, and *Steinernema riobrave* Rio strain were effective in controlling larvae and pupae at high concentrations (>200 infective juveniles per beetle, 50 infective juveniles per square cm) (Cabanillas and Elzen 2006). One study tested three commercially available strains of *Bacillus thuringiensis* against SHBs, but these strains were not effective (Buchholz et al. 2006). Only one protozoan pathogen has been

discovered but little is known about its life cycle and the effects that it has SHB (Wright and Steinkraus 2012).

Pathogens that attack SHBs may exist in the soil. Our objective in this study was to examine soil samples collected from around apiaries in Arkansas and use them in bioassays to determine if potential SHB pathogens were present. The data provides information on what pathogens are likely infecting SHB wandering-stage larvae, pupae, and emerging adults in the Arkansas soils.

**Materials and Methods.**

Soil samples were collected from 10 managed apiaries in Arkansas. A single hive was selected from each apiary. Soil was sampled from beneath the hive entrance and also adjacent to the colony (approximately one foot away from the hive entrance). Soil was taken at the surface and from 10 cm below the surface, about 100 ml of soil from each stratum. Thus, a single hive would have four soil samples associated with it. Soil samples were placed into quart-sized plastic zipper bags and kept cool in a cooler with ice packs.

Soil samples were collected at following Arkansas apiary coordinates on September 29th or 30th, 2011:

| | |
|---|---|
| DeWitt (site one): | 34°8'27.18"N, 91°17'33.54"W (DeWitt) |
| DeWitt (site two): | 34°5'15.73"N, 91°17'51.87"W (DeWitt) |
| Monticello site: | 33°35'54.57"N, 91°44'31.16"W (Monticello) |
| Carlisle site: | 34°45'36.67"N, 91°43'11.35"W (southeast of Carlisle) |
| Lepanto site: | 35°38'39.39"N, 90°20'56.52"W (northwest of Lepanto) |
| Gilmore site: | 35°43'85.04"N, 90°25'97.67"W (northeast of Gilmore) |
| Jonesboro site: | 35°52'7.19"N, 90°35'18.87"W (east of Jonesboro) |

Little Rock site: 34°45'42.08"N, 92°23'40.76"W (Little Rock)

W. Memphis (site one): 35°8'57.01"N, 90°19'34.42"W (west of W. Memphis)

W. Memphis (site two): 35°9'9.07"N, 90°21'50.04"W (west of W. Memphis)

Collected soil samples (four samples from each apiary) were mixed together in equal proportions so that each apiary had one combined sample. Each 100 mm plastic Petri dish contained 50 ml of soil, 3 ml of distilled water, and the following living insects from laboratory colonies: five mature wax moth larvae (*Galleria mellonella*), five wandering (post-feeding) SHB larvae, and five SHB adults. The Petri dishes were sealed with Parafilm® to prevent moisture loss and to keep insects from escaping. The sealed Petri dishes were then wrapped in aluminum foil to maintain complete darkness, inverted, and placed in an incubator at 23°C. This is the standard trapping technique for collecting entomopathogenic nematodes and fungi (Zimmerman 1986, Goettel and Inglis 1997).

The soil bioassays were started on March 28th, 2012. Three replicates were provided for each site. A total of 150 wax moth larvae, 150 SHB larvae, 150 SHB adults, and 30 Petri dishes were used for the bioassays. Petri dishes were examined every three days for 12 days. Cadavers were removed every three days and placed in a White trap at 23°C. White traps were examined every three days for fungal growth and the presence of nematodes for two weeks.

**Results.**

By day 12 of the experiment, 44 wax moths, eight SHB larvae, and five SHB adults had died (Table 1). Eight wax moth larvae, four SHB larvae, and one SHB adult could not be accounted for and were marked as "missing" (Table 1). Three SHB larvae escaped from one dish and were found dead in the incubator; these were marked as "escaped" but accounted for (Table 1).

No SHB cadavers appeared to have succumbed to entomopathogenic nematodes or fungi. No entomopathogenic fungi or nematodes were observed in the cadavers' White traps for up to two weeks after their deaths.

Of the 44 wax moths that died, 15 showed signs of *Beauveria*, a generalist entomopathogenic fungus, and three were infected by a *Steinernema spp.*, an entomopathogenic nematode. *Beauveria* was found at the following apiaries: DeWitt (site one), DeWitt (site two), Jonesboro, Little Rock, and West Memphis site one. The following locations had *Steinernema* present: DeWitt site one, Lepanto, and Jonesboro. The Jonesboro sample had both *Beauveria* and *Steinernema* present. All other cadavers either decomposed without visible signs of fungi/nematodes or were eventually colonized by *Aspergillus spp.*, a genus of saprophytic fungi.

**Discussion.**

Of the eight SHB larvae and one SHB adult that died, none showed signs of entomopathogenic fungi or nematodes. Because SHBs develop in the soil as part of their life cycle, the lack of infection seen in SHBs may be due to a natural resistance to soil pathogens. SHB larvae are considerably more sclerotized than wax moth larvae; this feature may aid in their resistance to entomopathogenic soil organisms. Wax moths develop in the maintained environment of a honey bee colony and are not adapted to life in the soil during any part of their development. For this reason, wax moths are highly susceptible to entomopathogenic soil organisms and are often used to "trap" for soil pathogens. There have been no investigations on the potential soil pathogens of SHBs in Africa. If pathogens exist, they may not have been transported with their hosts to new areas. It is possible that SHBs cause more damage in their introduced range because of their freedom from pathogens and natural enemies. Undoubtedly,

other factors also affect their damage capability, including climatic conditions, bee genetics, and soil type to name a few (Somerville 2003, Ellis et al. 2004b).

SHB pupae were not used in these bioassays, and it is possible that the pupae are more susceptible to soil pathogens than the wandering larvae and adults. Future work with SHBs and soil pathogens should include the use of SHB pupae as well.

Some wax moths and SHBs were "missing" from their dishes and could not be accounted for. The likelihood of escape was low; the three SHB larvae that escaped from one dish was due to the Parafilm® becoming unraveled. The lack of food may have resulted in cannibalism (Neumann et al. 2001) or the three day interval was long enough for cadavers to decompose beyond recognition. Some wax moth cadavers that were found were very soft and difficult to move without losing portions of their body. Given another day or two, these cadavers may have seemingly "vanished" and become a part of the moist soil in the dish.

It is clear from this data that small hive beetle larvae and adults are quite resistant to soil entomopathogens. Infections in *G. mellonella* show that fungal and nematode pathogens were present in the apiary soils, but no *A. tumida* became infected. This suggests that augmentative biological control by adding fungal or nematode pathogens to soil in apiaries is unlikely to control small hive beetles.

**Acknowledgements.**

Special thanks to Marco Cordero for his help in identifying nematodes and advice on how to make temporary mounts for photography.

**References.**

Buchholz, S., P. Neumann, K. Merkel, and H. R. Hepburn. 2006. Evaluation of *Bacillus thuringiensis* Berliner as an alternative control of small hive beetles, *Aethina tumida* Murray (Coleoptera: Nitidulidae). J. Pest. Sci. 79: 251-254.

Cabanillas, H. E., and P. J. Elzen. 2006. Infectivity of entomopathogenic nematodes (Steinernematidae and Heterorhabditidae) against the small hive beetle *Aethina tumida* (Coleoptera: Nitidulidae). J. Apic. Res. and Bee World 45(1): 49-50.

Ellis, J.D., I.H. Rong, M.P. Hill, H.R. Hepburn, P.J. Elzen. 2004a. The susceptibility of small hive beetle (*Aethina tumida* Murray) pupae to fungal pathogens. Am. Bee J. 144(6): 486-488.

Ellis, J. D., R. Hepbern, B. Luckman, and P. J. Elzen. 2004b. Effects of soil type, moisture, and density on pupation success of *Aethina tumida* (Coleoptera: Nitidulidae). Environ. Entomol. 33(4): 794-798.

Elzen, P. J., J. R. Baxter, D. Westervelt, C. Randall, and W. T. Wilson. 2000. A scientific note on observations of the small hive beetle, *Aethina tumida* Murray (Coleoptera, Nitidulidae), in Florida, USA. Apidologie 31: 593-594.

Goettel, M. S. and G. Douglas Inglis. 1997. Fungi: Hyphomycetes. *In* L. L. Lacey (Ed.) Manual of Techniques in Insect Pathology, Academic Press, San Diego.

Hood, W. M. M. 2004. The small hive beetle, *Aethina tumida*: a review. Bee World 85(3): 51-59.

Mürrle, T. M., P. Neumann, J. F. Dames, H. R. Hepburn, and M. P. Hill. 2006. Susceptibility of adult *Aethina tumida* (Coleoptera: Nitidulidae) to entomopathogenic fungi. J. Econ. Entomol. 99(1): 1-6.

Neumann, P., C. W. W. Pirk, H. R. Hepburn, A. J. Solbrig, F. L. W. Ratnieks, P. J. Elzen, and J. R. Baxter. 2001. Social encapsulation of beetle parasites by Cape honeybee colonies (*Apis mellifera capensis* Esch.). Naturwissenschaften 88: 214-216.

Richards, C. S., M. P. Hill, and J. F. Dames. 2005. The susceptibility of small hive beetle (*Aethina tumida* Murray) pupae to *Aspergillus niger* (van Tiegem) and *A. flavus* (Link:Grey). Am. Bee J. 145(9): 748-751.

Schmolke, M. D. 1974. A study of *Aethina tumida*: the small Hive Beetle, Project Report, University of Rhodesia, p. 178.

Torto, B., A. T. Fombong, R. T. Arbogast, and P. E. A. Teal. 2010. Monitoring *Aethina tumida* (Coleoptera: Nitidulidae) with baited bottom board traps: occurrence and seasonal abundance in honey bee colonies in Kenya. Environ. Entomol. 39(6): 1731-1736.

Wright, N. A., and D. C. Steinkraus. 2012. A scientific note on a protozoan pathogen of the small hive beetle. Apidologie. Available at (http://rd.springer.com/article/10.1007/s13592-012-0164-7).

Zimmerman, G. 1986. The '*Galleria* bait method' for detection of entomopathogenic fungi in soil. J. Appl. Entomol. 102: 213-215.

| AR County | Rep 1 | | | Rep 2 | | | Rep 3 | | |
|---|---|---|---|---|---|---|---|---|---|
| | SHB adult | SHB larvae | Wax moth | SHB adult | SHB larvae | Wax moth | SHB adult | SHB larvae | Wax moth |
| **DeWitt1** | 0 | 0 | 2 | 1 | 0 | 3 | 0 | 0 | 1 |
| **DeWitt2** | 0 | 0 | 4 | 0 | 0 | 2 (1) | 0 | 0 | 3 (1) |
| **Monticello** | 1 | 1 | 0 (1) | 0 | 0 | 1 | 0 | 1 | 0 |
| **Carlisle** | 0 | 0 | 1 | 0 | 1 | 1 | 0 | 0 | 2 |
| **Lepanto** | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 1 | 1 |
| **Gilmore** | 0 | 0 | 0 | 0 | 0 | 1 (1) | 0 | 1 | 1 (1) |
| **Jonesboro** | 0 | 0 | 3 (2) | 2 | 1 [3] | 2 | 0 | 0 | 1 (1) |
| **Little Rock** | 0 | 0 | 4 | 0 | 0 | 3 | 0 | 0 (1) | 3 |
| **W Memp1** | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 3 |
| **W Memp2** | 0 | 1 (1) | 0 | 0 (1) | 0 (2) | 0 | 0 | 0 | 1 |

Table 1. Number of deceased insects by day 12 of the bioassays. Numbers indicate cumulative dead, numbers in parentheses indicate missing insects that could not be account for, and numbers in brackets indicate escaped insects that were accounted for.

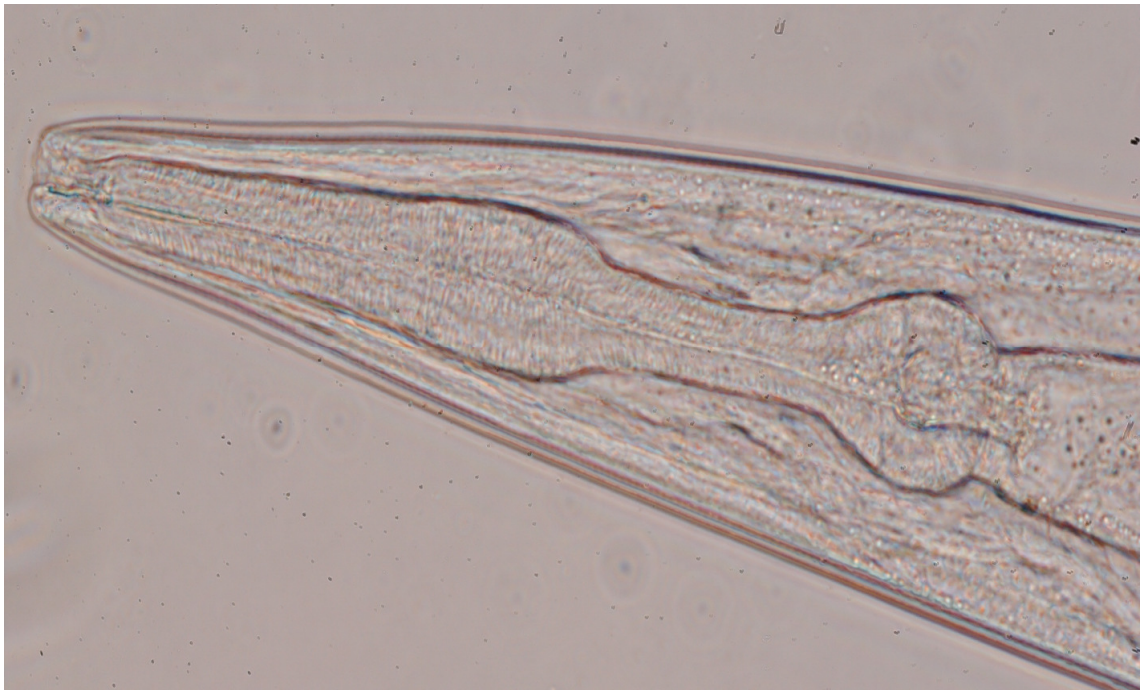

Fig 1. The anterior end of a *Steinernema* nematode from DeWitt (site one).

Fig 2. An adult female *Steinernema* from DeWitt (site one) with young nematodes developing inside. *Steinernema* are oviparous or ovoviviparous and do not lay eggs.

**Conclusion.**

The development of a cost-effective rearing technique saved time and hassle, otherwise, beetles would have had to been harvested from infested honey bee colonies each time they were needed for research or photography. The accessibility of these beetles then allowed for the development of dissection techniques and the inspection of the internal organs so that healthy specimens could be distinguished from infected ones. The photographs of the external morphology of the small hive beetle were all taken from lab-reared specimens. Beetles and their larvae were also needed in ample supply for the soil bioassays, in which 150 adults and larvae were used to determine their susceptibility to local soil pathogens.