Evaluation of the Efficacy of Small Hive Beetle (*Aethina tumida* Murray) Baits and Lures

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Summary

Two experiments were conducted to evaluate the attractiveness of a lure being developed by the Food and Environment Research Agency (=UK lure) to adult small hive beetles (SHB). Experiment 1 compared the UK lure with: USDA fermented pollen dough, banana scent, apple cider vinegar and their combinations while Experiment 2 included: USDA fermented pollen dough, slow-released ethanol (SRE), SuperBoost (SBt), apple cider vinegar and combinations of these products. Overall, the USDA pollen dough inoculated with the yeast, *Kodamaea ohmeri*, was the most attractive bait for adult SHBs especially when used in combination with SBt (the brood pheromone), and SRE. It is possible that the volatiles associated with the yeast had a synergistic effect when combined with the several compounds that made up SBt. Also, the possible increase in the amount of alcohol from both SRE and fermenting pollen dough significantly improved trap catch. The unattractiveness of the UK lure to SHB may be due to an insufficient amount used per trap exacerbated by its rapid evaporation rate. Further development work to decrease the evaporation rate of the UK lure is recommended.

Keywords small hive beetle/lure/bait/trap/efficacy

Introduction

In the United States, the small hive beetle (SHB) has killed many colonies since its detection in 1998 in Florida (Elzen *et al.* 1999). The SHB has since expanded its distribution with problems being more evident in the southern-eastern region where beetles are more abundant (Neumann and Elzen 2004). This abundance is a consequence of hot weather which is known to shorten SHB developmental time, and hence more generations per year (de Guzman and Frake 2007). Nevertheless, honey bee stock is reported to influence the number of SHB in honey bee colonies (Frake *et al.* 2009, de Guzman *et al.* 2010).

In the USA coumaphos strips or CheckMite+® (stapled to corrugated board for use inside the colonies) and GardStar® (a permethrin soil drench for use in front of colonies) are currently recommended (by whom?) for managing SHB populations but neither is particularly effective. Further, the inappropriate use of insecticides can have negative consequences such as the development of resistant SHB populations, high toxicity to honey bees and contamination of honey and other hive products.

Traps baited with attractants are not only used for the detection and survey of insect populations, but in some cases are also used for controlling them. At present, several designs of in-hive SHB traps are commercially available. However, the efficiency of these traps relies significantly on the attractiveness of baits or lures. Different attractants have been tested in several trapping experiments. The combination of hive products such as honey, pollen, combs, brood and adult bees are very attractive to SHB (Elzen *et al.* 1999). Pollen dough inoculated with the yeast, *Kodamaea ohmeri*, was shown to be effective in attracting adult SHB (Arbogast *et al.* 2007), as was apple cider (Nolan and Hood 2008). Although attractive to SHB, traps containing fermented pollen dough were unable to lure a majority of SHB from inside colonies to traps located outside the hive (de Guzman *et al.* 2011). In order to maximize trap catch, a better bait or lure is needed. This study was conducted to evaluate the efficiency of a lure (referred to hereafter as UK lure) being developed by the Food and Environmental Research Agency (FERA) in the United Kingdom to monitor small hive beetle numbers.

Materials and Methods

To trap beetles inside the colonies, the Hood SHB traps Nolan and Hood (2008) were used. This trap consists of three compartments. The lures or baits were placed at the middle compartment, and the two side compartments received vegetable oil (about 30 ml) to prevent adult beetles from escaping. Each trap was attached to the bottom of a frame having no comb or foundation as described by Nolan and Hood (2008).

Experiment 1

We determined the attractiveness of the UK lure to adult SHB inside nucleus colonies. The following baits and lures were used: USDA pollen dough, USDA pollen dough + UK lure, banana scent (Nature's garden), USDA pollen dough + banana scent, UK lure + banana scent, and apple cider vinegar (White House). No "unbaited control check" colonies were monitored for this study. Instead, we used the USDA pollen dough and apple cider as reference standards since they known to be attractive to SHB adults (Arbogast *et al.* 2007, Nolan and Hood 2008). Corrugated plastic board stapled with coumaphos strip was also included because it is the recommended control measure for SHB in the U.S.

The UK lure which was an alcohol-based lure was prepared according to the instructions provided by FERA. About 1 ml of the UK lure was placed in an eppendorf vial, hung inside each trap and replaced every day. Similarly, 1 ml of the banana scent placed in an eppendorf vial was used per trap. For the pollen dough baits, pollen patties (4% pollen) were inoculated with the yeast *K. ohmeri* as described by Arbogast *et al.* (2007); about 1/4 cup placed in a mesh bag was used per trap. About 30 ml apple cider vinegar was used per trap.

For each treatment, three nucleus colonies with three medium frames were used for a total of 27 colonies. In addition, three nucleus colonies that did not receive any trap or treatment were also monitored for the presence of adult beetles to determine if the presence of baits encourages SHB invasion into honey bee colonies. Each colony was headed by a mated queen of mixed origin. All colonies were sitting on hive stands about 2½ ft (76 cm) above ground. This means that adult SHB (whether emerging from the soil or invading from other colonies or apiaries) would have to fly to invade any of the test colonies. In Louisiana, the population of SHBs in the colonies is known to increase in the fall months (de Guzman *et al.* 2010). Hence, the traps were installed in September 1, 2009 and were monitored everyday for one week. The initial and final infestations of SHB per colony were determined by examining individual frames, all walls of the hive box and hive cover for the presence of SHB (de Guzman *et al.* 2006).

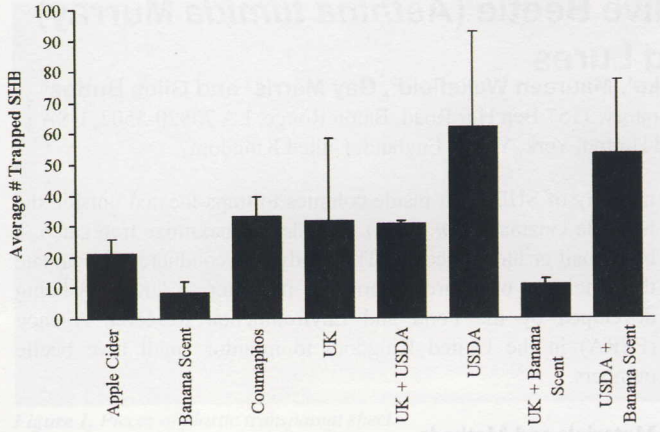

***Figure 1**. Number of adult beetles (Mean ± SE) caught in Hood beetle traps baited with different lures. Bars without letters are not significantly different (P > 0.05, Tukey test).*

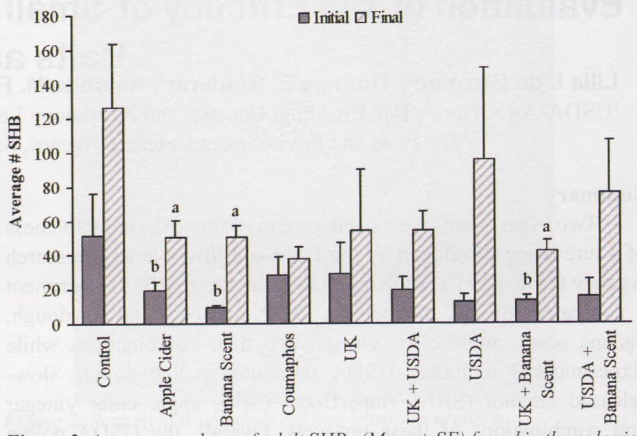

***Figure 2**. Average numbers of adult SHBs (Mean ± SE) found in the colonies before baited traps were installed and at the end of the experiment. For each lure or bait, bars without letters are not significantly different (P > 0.05, Tukey test).*

Experiment 2

Hood SHB traps were installed on October 15, 2009 using 55 three-frame nucleus colonies headed by mated queens of mixed origin. This experiment used similar methods as those of Experiment 1 however, a different array of potential lures was evaluated using five colonies per lure. The lures evaluated were: UK lure, USDA pollen dough, USDA pollen dough + UK lure, SuperBoost (SBt), USDA + SBt, UK lure + SBt, USDA + UK lure + SBt, Slow-release ethanol (SRE), USDA pollen dough + SRE, and USDA pollen dough + UK lure + SRE. SuperBoost (Contech), a honey bee brood pheromone which is made of several compounds (Pankiw *et al.* 2010), was included in this study to determine if brood pheromone will attract more beetles since brood is an important part of SHB's diet. Since infestation by SHB is always characterized by the fermentation of hive materials and since SHB were attracted to ethanol in the UK under laboratory conditions (Maureen Wakefield, Pers. Com.), the SRE was included in the experiment. Moreover, ethanol has been known to affect infestations of other beetles; induce infestation of spruce beetles (Moeck 1981), and attract scolytid beetles (Kelsey and Joseph 2001). As in Experiment 1, the USDA pollen dough bait served as the standard. In addition, five control colonies (without traps) were monitored to determine whether or not the presence of bait in the colonies will attract more beetles into the colonies. Traps and control hives were examined daily. At that time, UK lure was replaced because of its quick evaporation rate. Observations were made for two weeks. Weather data were obtained from the Louisiana Agriclimatic Information website (**http://www.lsuagcenter.com/weather**).

Data analyses

Data on the number of trapped beetles were analyzed by trial period using Proc Mixed in a one-way analysis of variance (ANOVA) with treatment as the variable to determine differences among the treatment groups. Before analysis, data were transformed using a square-root transformation to approximate normality. Data on the initial and final (final number of living SHB in the colonies plus the total number of trapped beetles) were analyzed by treatment using t-tests (SAS Institute 2008).

Results and Discussion

Number of adult small hive beetle trapped

For Experiment 1, a total of 752 adult beetles were trapped across all treatments during a week of trapping. This high number of trapped beetles may be influenced by warm temperature during this time. The average maximum and minimum air temperatures were about 32°C and 21°C, respectively. Overall, no significant differences in trap catches ($F=1.56$, $P = 0.216$) were detected among the different lures (Figure 1). This lack of difference may be due to a combination of the high variation in the number of trapped beetles that we observed among colonies within most of the treatment groups and a small sample size. There were only 30 colonies used in Experiment 1. Nevertheless, all traps caught beetles indicating that all lures were at least somewhat attractive to SHB.

In a comparison between the initial and final (number of live beetles in the colony plus total trapped SHBs) numbers of SHBs for each treatment, all treatment groups including the control colonies (without baited traps) had more beetles after the baited traps were installed (Figure 2). However, only the Apple cider ($t = 2.79$, $P = 0.05$), banana scent ($t = 7.03$, $P = 0.002$), and UK + banana scent ($t = 4.38$, $P = 0.012$) groups showed significant differences. This significant increase in the number of SHBs may suggest that the presence of Apple cider and banana scent lures in the colonies may have encouraged more beetles to invade the colonies. The Apple cider has been known to be attractive to SHB adults (Nolan and Hood 2008). The attraction of SHBs to colonies having banana scent-baited traps may be related to the association of isopentyl acetate with both the odor of bananas and honey bee alarm pheromone. The yeast, *K. ohmeri,* which was isolated from SHBs, is known to be associated (produced and released) with the alarm pheromone (Torto *et al.* 2007). In addition, the banana scent may have improved the attractiveness of the UK lure.

Number of adult small hive beetle trapped

For Experiment 2, a total of 369 beetles were trapped from nearly two weeks of trapping. Despite the longer trapping period, this number was lower than the number of beetles caught in Experiment 1. This disparity may be due to cooler temperature during Experiment 2 since the average maximum air temperature was about 23°C with a minimum average of about 11°C, about 10°C lower than that of Experiment 1.

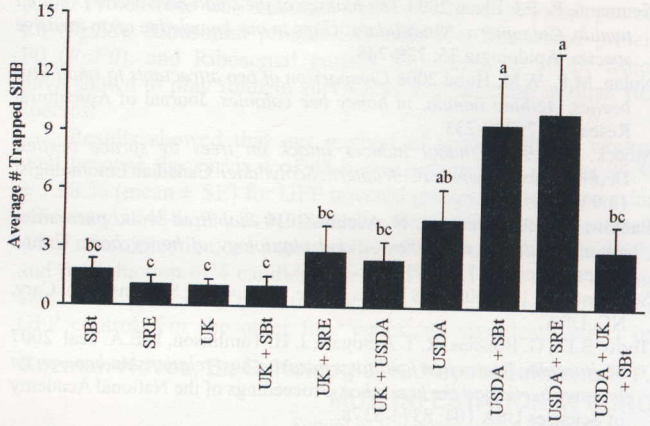

***Figure 3**. Number of adult beetles (Mean ± SE) caught in Hood SHB traps baited with different lures. Bars with the same letters are not significantly different (P > 0.05, Tukey test).*

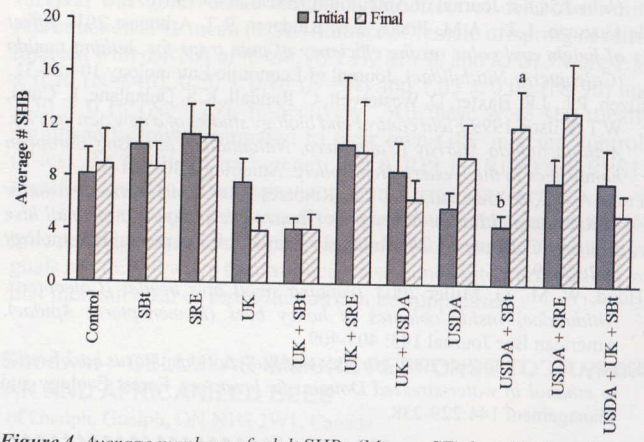

***Figure 4**. Average numbers of adult SHBs (Mean ± SE) found in the colonies before baited traps were installed and at the end of the experiment. For each lure or bait, bars without letters are not significantly different (P > 0.05, Tukey test).*

Analysis of the number of beetles trapped showed a significant difference ($F = 4.57$, $P < 0.0001$) among the treatment groups (Figure 3). The traps baited with USDA + SRE and USDA + SBt produced the highest numbers of trapped adult beetles but the numbers captured were not significantly different from those captured in traps baited with fermented USDA pollen dough alone. The lowest numbers of trapped beetles were recorded in traps baited with SRE and UK but were not different from traps baited with SBt, UK + SRE, UK + USDA and USDA+ UK + SBt. This study showed that the addition of SRE or SBt increased the attractiveness of the USDA fermented pollen dough. It is possible that the alcohol, as a product of fermentation triggered by the yeast, in the USDA pollen dough was insufficient and that the addition of SRE improved its attractiveness. With SBt, it is possible that the different compounds that made the pheromone may have had a synergistic effect when combined with the volatiles produced by the yeast. *K. ohmeri* has been documented to be associated with several pheromones most notably the alarm pheromone of honey bees (Torto *et al.* 2007).

After the installation of baited traps, the number of beetles (final live beetles plus total trapped SHBs) was similar to the initial number in nearly all treatment groups (Figure 4). Hence, the presence of baits inside the colonies did not attract further invasion of beetles with the exception of the USDA + SBt groups. Most of these beetles were recovered from the trap. Again, this observation may indicate synergistic effect of USDA pollen dough when combined with SBt. Further, the discrepancy between our results in Trial 1 with that of Trial 2 may be due to the presence of the banana scent and apple cider in Trial 1 which may act as attractants with long-range volatility.

There were 10 colonies that were lost from either absconding or loss of queens during the experiment. However, there was no indication that colony loss was caused by the use of particular bait. The colonies were from the following groups: 2 unbaited control, 3 SBt, 1 UK, 3 UK + SRE, and 1 USDA + SBt. These small colonies may have been lost because of cool weather. The lowest temperature during the experiment was 5°C.

Although SHBs have a strong attraction to fermenting materials, the UK lure which is alcohol-based failed to consistently attract adult beetles. This observation was in agreement with the findings of Hood and Miller (2003) regarding the unattractiveness of alcohol (95% ethyl alcohol) to SHBs. This unattractiveness of the UK lure, however, may result in part from the recommended 1 ml of lure per trap being small and evaporating quickly. SHBs are excellent fliers and they are known to locate hosts or actively forage for food by smell. But the 1 ml lure may have already evaporated and thus, have lost effectiveness long before each trap was serviced the following day. Despite the cool weather, all vials were empty during each day of examination. Further development to decrease the evaporation rate of the UK lure may improve its attractiveness.

Conclusions and Recommendations

Lures and baits are used to increase capture rate of target insect pests. In this study, the USDA pollen dough inoculated with the yeast, *Kodamaea ohmeri*, was the most attractive bait for adult SHBs especially when used in combination with SBt, and SRE. The volatiles associated with the yeast may have a synergistic effect when combined with the compounds that made up the brood pheromone (SBt). It is also possible that the increase in the amount of alcohol from both SRE and fermenting pollen dough significantly improved trap catch. Inevitably, there are many problems associated with trapping SHBs. Capture rate depends on the abundance of beetles at the time of trapping, volatility and attractancy of the lures. In addition, temperature may not only alter the beetles' behavior but also the performance of the baits and lures. The unattractiveness of the UK lure to SHB may be due to an insufficient amount used per trap exacerbated by its rapid evaporation rate. Hence, further development work to decrease the evaporation rate of the UK lure is needed.

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Saraithong[a], P., Y. Li[b] & P. Chantawannakul[a,c] – BACTERIAL COMMUNITY STRUCTURE IN THE MIDGUT OF *APIS DORSATA* WORKERS IN THAILAND

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Asian giant honey bees are vital honey produces and pollinators of cultivated crops and wild plants. Relationships between gut microorganisms and honey bees are essential for maintaining proper nutrition and immunity. This study examined the bacterial community structures in the midgut of the Asian giant honey (*A. dorsata*) workers collected from two locations in Chiang Mai, Thailand. A total of 180 workers from six colonies were collected at different geographic sites in northern Thailand. Polymerase chain reaction-based denaturing gradient gel electrophoresis (PCR-DGGE) is a cultivation-independent molecular fingerprinting technique that allows the assessment of the predominant bacteria species present in bee midguts. The result showed the mean species richness and the Shannon index differed between colonies but nor by locations. Bacterial DNA profiles had similar patterns in individual colonies which differed amongst the replicate colonies, but was not affected by graphical location. Sequence analysis of DGGE products revealed evidence for core bacteria of the genera *Gammaproteobacteria* and *Firmicutes.* Although core bacteria existed in both populations, specific bacterial species were observed for each colony and site.

Milbrath, M.O.[a], X.B. Xie[a, b], & Z. Y. Huang[a] CARBON DIOXIDE ANESTHESIA AFFECTS MORTALITY OF *NOSEMA CERANAE* INFECTED HONEY BEES

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We have known for almost a century that the microsporidia *Nosema apis* is a serious parasite of the Western honey bee (*Apis mellifera*). Only recently, we have identified that a related microsporida, *Nosema ceranae,* has transferred from its original host, the Eastern honey bee, and is causing serious infection in *A. mellifera* as well. The full effects of *Nosema ceranae* infection in this new host remain unknown. Numerous studies have examined mortality after experimental infection with *N. ceranae*, but they have had highly variable results. One reason for this variation may be differences in experimental techniques. We examined one technique, CO_2 anesthesia, that may affect honey bee survival in the presence of nosema infection. We hypothesized that the use of CO_2 anesthesia when infecting bees would reduce survival. We used four treatments (Control, Nosema only, CO_2 anesthesia only, CO_2 anesthesia /Nosema), repeating the experiment with three colonies. We found that bees infected with Nosema spores alone had significantly lower survival than control bees (median survival = 21 days and 23 days, respectively), and that CO_2 anesthesia had a greater effect on survival than nosema infection alone. Bees infected using CO_2 anesthesia survived for significantly shorter times, regardless of their infection status (median survival = 18 days for both groups). Interestingly, bees infected using CO_2 had significantly fewer spores than bees infected without anesthesia. These results indicate differences in honey bee mortality experiments may be due in part to experimental technique. Overall, our survival rates were higher than these previous nosema mortality experiments, indicating that variation in honey bee resistance to nosema may be an important factor in determining survival after being infected with this parasite.

Xie, X.B.[a, b], G.W. Bian[b], Z. Xi[b], & Z.Y. Huang[b]-USING RNAi TO HUNT FOR GENES IMPORTANT FOR *VARROA* SURVIVAL AND REPRODUCTION.

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The varroa mite, *Varroa destructor,* is the worst pest of the Western honey bee (*Apis mellifera*) and responsible for declines in honey bee populations worldwide. In this study we used RNA interference (RNAi) technology to disrupt the life cycle of varroa mites by either causing death or causing a reduction in reproduction. We searched for gene orthologs in the newly established varroa mite genome (http://www.ncbi.nlm.nih.gov/genome/?term=varroa%20destructor). We tested the genes of Daughterless