Laboratory comparison of soil treatments for the control of the small hive beetle (*Aethina tumida*)

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Introduction

The small hive beetle (SHB), *Aethina tumida* Murray (Figure 1 A,B), is a honey bee pest of increasing concern to beekeepers, especially in the southern United States. The majority of their damage is caused by actively feeding larvae. (Figure 1. C) When they stop feeding, matured larvae leave the hive and pupate in the soil. SHB spends about 75% of their developmental time in the soil wandering to find ideal pupation locations, and during pupation itself which takes about 5 to 10 days depending upon the temperature (de Guzman and Frake 2007).

There are two chemicals (Coumaphos and Gardstar) available for SHB control. While Coumaphos is used inside the hive, Gardstar is a soil drench. Nevertheless, the efficacy of both chemicals has been highly variable. Hence, there is a need to find additional methods to mitigate SHB problems.

Overall Objectives

The overall goal was to use a simple in lab bioassay system to test multiple commercially available small hive beetle control methods for survivability and active ingredient repellency to soil treatments.

Specific objectives

- To compare the efficacy of three commercially available inorganic materials (lime, diatomaceous earth), OII-Y2, and the permethrin insecticide, GardStar, against SHB.
- To test the efficacy of different concentrations of GardStar
- To evaluate repellant property of GardStar treatment.

Materials and Methods

Beetles

To obtain SHB larvae of the same age, SHB was reared in the laboratory as described by de Guzman and Frake (2007). In brief, adult beetles collected from infested honey bee colonies were placed in chambers containing bee pupae, honey, and pollen for egg-laying. Wet cotton balls (in honey solution) provided humidity in the rearing box. Eggs laid on the same day were isolated and maintained until hatching and reaching the "wandering" phase or when larvae stopped feeding.

Soil

Soil used in the experiments was collected from one of our laboratory apiaries, sifted, and then baked in an oven at 204°C for 30 mins to sterilize.

Treatments

•GardStar (40% EC): active ingredient = Permethrin (synthetic pyrethroid), used at label rate (0.05% active ingredient, 21oz of liquid per hive, treating 2-2.5 sq feet of soil at hive entrance), scaled down for use in small containers (2 ml of 0.05% solution per 1 sq inch surface area)

•OII-Y2 (Organism II-YS): active ingredients = chitosan and yucca plant extracts, used at label rate (0.025%, 2 qt into 20 gal of water per acre) scaled down for use in small containers (0.124 ml of 0.025% solution per 1 sq inch surface area)

•Diatomaceous Earth: 1 gm per 1 sq inch of surface area

•Lime: 1 gm per 1 sq inch of surface area

Survival Bioassays

Falcon tubes (50 ml) with small air holes punched through the lids were filled with 40 g of sterilized soil then moistened with 10 ml sterile H_20 (Figure 2 B). GardStar (GS), OII-Y2, Lime or Diatomacious earth were added to the top of the soil. A non-treated control group contained only moistened soil. A separate experiment assessed the effectiveness of different dilutions of GardStar (LR or 1/10, 1/100 and 1/1000 dilutions). Ten third instar larvae were placed onto the top of the soil and larvae were allowed to burrow. Tubes were placed in an incubator and inspected daily for 4 weeks and emergence of adult beetles was noted. Water was added when needed to prevent desiccation. Each treatment was replicated 20 times. One tube represented one replication.

GardStar repellency bioassay

To measure the repellency of the GS treatment, 1qt square plastic containers were filled with 600 g of moistened soil (Figure 2 C). Each container was equally divided into two sections using a plastic divider which allowed application of two treatment types per container. Each container randomly received one of the following treatment combinations: control/control; GS/control; GS/GS at the label rate. After each section received the assigned treatment, the divider was removed to create a "choice pupation site" for SHB larvae. Thereafter, 20 third instar larvae were introduced at the middle of each container. The containers were covered and placed in an incubator at 34°C. After four days the containers were removed, redivided with plastic, and the soil from each side was separately sifted to count the number of larvae.

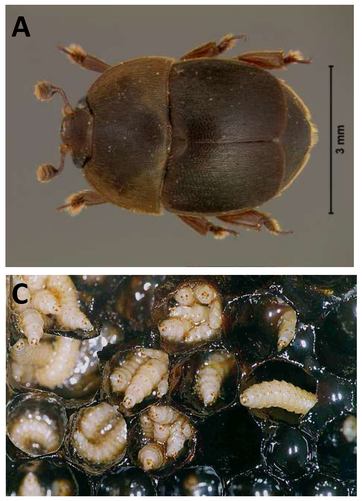

Figure 1. Small hive beetle life stages A) adult dorsal Photo by Lyle J. Buss, University of Florida B) adult ventral Photo by Josephine Ratikan, University of Florida
C) larvae feeding in honey bee comb

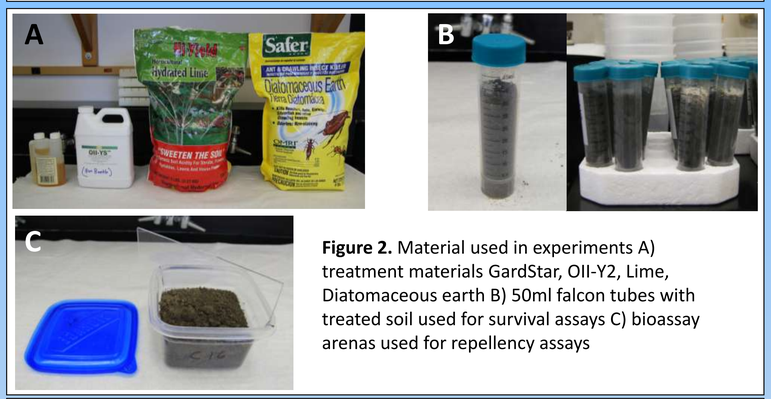

Figure 2. Material used in experiments A) treatment materials GardStar, OII-Y2, Lime, Diatomaceous earth B) 50ml falcon tubes with treated soil used for survival assays C) bioassay arenas used for repellency assays

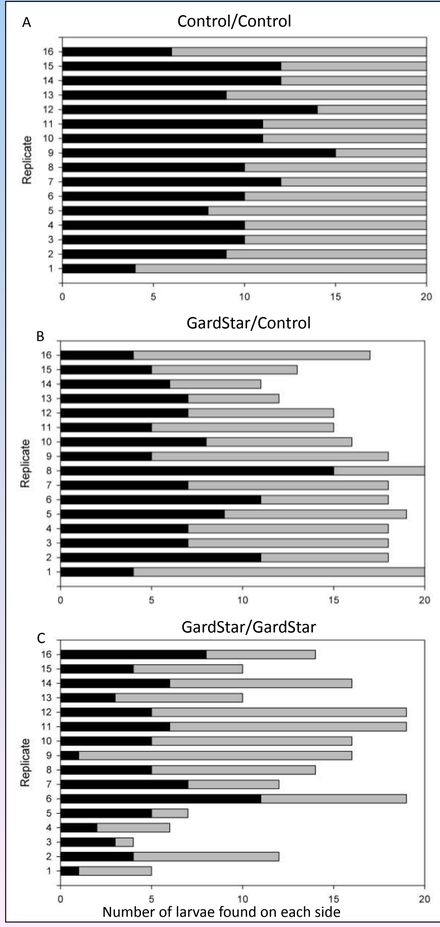

Figure 5. Soil repellency assay was used to determine if GS was acting as a repellant to SHB larvae. **Black** bars represent Side A, grey bars represent side B A) Control/Control- no treatments were put on either side. B) GardStar/Control- GardStar was put on one side while nothing was put on the other. C) GardStar/GardStar- GardStar was placed on both sides of the container. Twenty third instar larvae were placed in the middle of each container. Four days later the number of larvae on each side was counted.

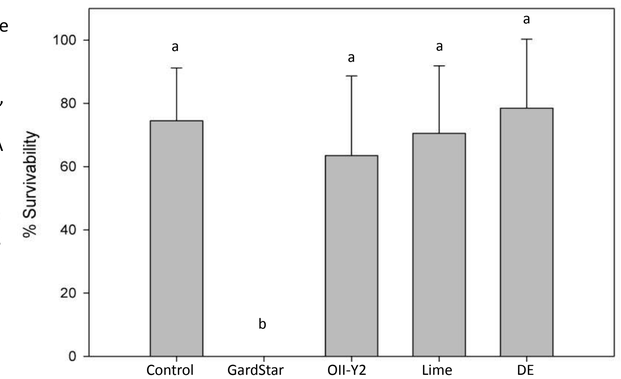

Figure 3. Small hive beetle survival in response to different soil treatments (Control, GardStar, OII-Y2, Lime, Diatomaceous Earth). A two-way ANOVA was used to detect significant differences followed by a Tukeys HSD test for separating means by treatment ($p<0.05$) (ANOVA,d.f. 4,99; F = 57.392; $P < 0.0001$).

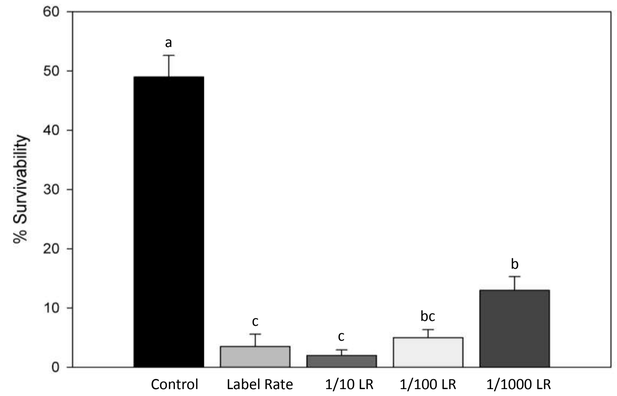

Figure 4. Effect of different concentrations of GardStar on SHB survival. A two-way ANOVA was used to detect significant differences followed by a Tukeys HSD test for separating means by treatment ($p<0.05$). (ANOVA; d.f. 4,99; F = 76.58; $P < 0.0001$)

Results and Discussion

Survival Assays

Results showed that the permethrin (GardStar) was highly effective at killing SHB larvae, while the other treatments had no significant effect on survivability in this bioassay (Figure 3). During the experiment, it was noticed that the larvae were not burrowing into the soil, suggesting that the permethrin was acting as a repellent. The lime and diatomaceous earth was added as a layer on top of the soil, perhaps if they were mixed into the soil they would be more effective (Buchholz et al 2009). Tests are currently being conducted to test this hypothesis. The OIlY2 is designed to be an adjuvant for agricultural applications. Organism II-YS contains a combination of chitosan and yucca plant extracts and is used in pasture and crop applications. The proposed mode of action is the increased feeding of soil micro-organisms, therefore increasing their ability to digest insect chitin. But, because we heated the soil we most likely killed all of the microorganisms designed to feed off of the OlIY2. Tests are currently being conducted to test this hypothesis.

GS concentration Assay

Analysis on the survival of SHB larvae among different concentrations of GardStar indicated that even at reduced concentrations, GS was still effective at controlling beetles (Figure 4). However, there was an increased mortality in the control treatments as compared to the previous experiments.

Repellency Assay

Since we observed an increased mortality and a potential repellant effect from the GS treatment in survival assays, we further determined whether or not GS has a repellant property. In a pupation location choice test, a two-tailed *t*-test indicated that there were no differences in the control/control ($P = 0.788$) or GS/control ($P = 0.193$) treatments. However, a significant shift in the GS/GS ($P = 0.025$) treatments was detected (Figure 5). Although all of the introduced larvae were recovered from the control group 100% (20.0±0), a significantly less amount was recovered from the GS/Control 81.3% (16.6±0.6) and GS/GS 62.1% (12.44±1.13) treatments (ANOVA; d.f. 2,47; F =20.93; $P < 0.0001$). This observation suggests that even with sealed containers either the larvae were able to escape, or were overlooked when the soil samples were examined. There were fewer larvae in soil treated with an increasing amount of GS, which may indicate a repellency effect.

Overall small hive beetles are a small but potentially serious problems for beekeepers and there is a need for safe efficient control methods. The results from these experiments demonstrated the effectiveness of the permethrin insecticide against SHB. However, there are some indications of its repellency, which may be an important concern when treating apiaries for SHB control.