Efficacy of Entomopathogenic Fungi in Controlling the Small Hive Beetle; a Destructive and Invasive Pest of Honey Bee Colonies

Final Report for GS11-100

Project Type: Graduate Student
Funds awarded in 2011: $9,996.00
Projected End Date: 12/31/2011
Grant Recipient: Florida A&M University
Region: Southern
State: Florida
Graduate Student:

Saundra Wheeler

Major Professor:

Lambert Kanga
Florida A&M University

Project Information

Summary:

A comparison of Metarhizium anisopliae 5680 to Metarhizium anisopliae 3020 in field trials determined that the Metarhizium anisopliae 5680 appeared to be a sustainable and cost effective option for beekeepers. Although a higher dose of Metarhizium anisopliae 3020 was used, M. anisopliae 5680 provided better control of small hive beetle populations while maintaining hive health in the 73- day study. DNA fingerprinting of unknown pathogens retrieved from cadavers collected during the field trials indicated saprophytic fungi and potential microbial control agents.

Introduction

Statement of Problem, Rational and Justification

The purpose of this project is to evaluate the fungal pathogen (Metarhizium anisopliae) against the small hive beetle Aethina tumida for the development of a sustainable approach to controlling this invasive pest species in hives in Florida. The European honey bee, Apis mellifera is the most economically important insect of agricultural crops worldwide, not only for honey production, but also for crop pollination (Sweetser, 2009). In the U.S., bee pollination of agricultural crops accounts for about one-third of the U.S. diet (Sanford, 1999). The monetary value of honey bees as pollinators in the United States is estimated at about $14.6 billion annually (Morse and Calderone, 2000). This estimate includes the benefit of production attributable to honey bees in terms of the yield increase and achieved quality from honey bee pollination, including the indirect benefits of bee pollination required for seed production of some crops. Many agricultural crops are almost

totally dependent on honey bee pollination, including almonds, apples, avocados, blueberries, cranberries, cherries, kiwi fruit, macadamia nuts, asparagus, legume seeds, pumpkins, squash, and sunflowers. Honey bees also contribute to biodiversity by pollinating other wild types of plants, and honey products and bee venom are important in health food and alternative medicine. However, honey bee colonies in the U.S. have declined drastically over the past few years (Ellis et al., 2004). A new and invasive pest species, Aethina tumida (small hive beetle) is responsible for substantial loss of honey bee colonies over the last four years. Adults are long lived; surviving up to 181 days and females may lay up to 2000 eggs in their life time (Lundie, 1940). Research has found that this pest can produce up to five generations per year (Lundie, 1940). The rapid spread and high reproductive potential of the SHB both within colonies as well as in stored products coupled with the ability to hibernate in honey bee clusters make it a serious threat of apiculture (Hood, 2000). Studies also show that SHB are potential biological vectors for honey bee viruses which also make them a threat to apiculture (Eyer, 2008) Substantial damage to the honey bee colonies is caused mainly by the feeding of larvae of SHB on honey, pollen and live brood. They also tunnel and pierce wax combs; defecate in and ferment stored honey causing it to weep and froth away from the cells (vanEngelsdorp et al., 2004). It only takes two or three beetles to cause severe damage to a pile of supers (Lundie, 1940). As a result, there is a drastic reduction of all feral and managed honey bee populations and this situation threatens honey production, as well as the crops that rely on honey bees for pollination. Currently, there is no effective control measure for SHB even with the emergency use permit for in-hive application of Coumaphos (Elzen et al., 1999). In addition, the efficacy of soil drench under infested colonies with permethrin (GardStar 40% EC) is dependent on the timing of the applications (Neumann, 2004).

Although insecticides have shown a substantial efficacy in managing pest, they have also raised concern for environmental protection. Over use of insecticides can cause resistance or lead to a resurgence of a pest (Hood, 2010). Current research now focuses on methods of biological control. Biological control involves effective use of parasitoids, predators, pathogens, antagonists, or competitor populations to suppress a pest population making it less abundant and thus less damaging (Driesche and Bellows, 1996). Traditionally, it is the manipulated reduction of an insect population by natural enemies, parasites, and pathogens. Further development of biological control agents will lead to the reduction of chemical applications in honey production, the increase in the health of honey bee colonies, and the enhancement of crop pollinations. The evaluation of entomopathogenic fungi against SHB offers relevant biological control option for a successful pest management program. Some entomopathogenic fungi have shown virulence and specificity against a range of insect hosts. Entomopathogenic fungi can be found in various places and can be isolated from insects, soil, and other substrates. Metarhizium anisopliae and Beauveria bassiana, have been used to effectively control other species of Coleoptera, thus these fungi could provide new avenues for an environmentally sound management of the small hive beetle populations. Both M. anisopliae and B. bassiana were found to be harmless to honey bees (Kanga et al., 2003) Scientists have demonstrated that honey bees could be used to vector fungal control agents for control of certain coleopteran (Shipp et al., 2008) Successful control could be achieved by exposing pest populations to the most efficient fungi at the optimal concentration. Previous laboratory studies have shown that Metarhizium anisopliae and Beauveria bassiana could be used as biological control agents against the small hive beetle in soil treatments (Somorin, 2009). That same study indicated that the small hive beetle was more susceptible to M. anisopliae (Somorin, 2009).This project will consist of applied research that will

investigate the feasibility of the use of M. anisopliae in effective concentrations and the optimal dispersal of entomopathogenic propagules in the field.

Determination of Infection

To determine if mortality was due to fungal infection, dead SHB (larvae or adults) were collected from the treatments and the controls. The cadavers were collected from the hive or lab rearing jars then surface-sterilized, by immersing in a sterilant disinfectant, Expor (Expor, Alcide, Redmond, WA) for 3 minutes and rinsed once with 95% ethanol for 2 minutes. The cadavers were then transferred with soft wide-tip forceps to a sterile paper to dry the ethanol, and later the specimens were plated on PDA (Potato Dextrose Agar) in a sterile hood then incubated at 27± 1 ºC for 4-10 days. Cadavers retrieved from beetle traps were plated directly on to PDA media and incubated. The Petri dishes were sealed with parafilm strips and labeled with date and description prior to incubation. Dead SHB were observed daily for the presence of external fungal hyphae. Only SHB that showed fungal growth were considered to have died of infection and used in the data analysis.

- Bioassay preparation
- Recording SHB Mortality
- Treatment 3 Colonies
- Treatment 2 Colonies
- Treatment 1 Colonies

Project Objectives:

Chemical control measures are currently being used against the small hive beetle but they are inefficient and unsustainable. Therefore, the main objective if this study was to investigate biological control of the small hive beetle. The specific objective of this study was:

i. To determine the impact of M. anisopliae on small hive beetle survival and development in soil treated bioassays in the field.

Research

Materials and methods:

Bioassays with fungal pathogens

Field trials were conducted at the FAMU Research and Cooperative Extension Center, Quincy, FL from August to October, 2012 which included about three small hive beetle life cycles. To conduct the field experiments, thirty honey bee colonies were divided into three groups of ten; groups were at least 100 m apart to minimize the drifting of adult bees between colonies. Each group of colonies was randomly assigned a treatment. In treatment 1, ten metallic pans were covered with mixture of beach sand, 10.0 g of Metarhizium anisopliae 3020 dry fungal spores, and Crisco vegetable shortening; the pans were then placed underneath each assigned colony. Treatment 2 was similar to treatment 1, but in this case 2.0 g Metarhizium anisopliae 5680 dry fungal spores was mixed with the sand and the shortening. Colonies in treatment 3 served as controls and consisted of 10 pans covered with sand. The density of the populations of SHB in each honey bee colony was monitored by beetle blaster traps at time intervals during the experimental period.

The mortality of each honey bee was recorded during the course of the 73-day period of the experiments.

Determination of Infection

To determine if mortality was due to fungal infection, dead SHB (larvae or adults) were collected from the treatments and the controls. Cadavers retrieved from beetle traps were plated directly on to PDA media and incubated. The Petri dishes were sealed with parafilm strips and labeled with date and description prior to incubation. Dead SHB were observed daily for the presence of external fungal hyphae. Only SHB that showed fungal growth were considered to have died of infection and used in the data analysis.

Data Collection

To monitor the populations of SHB in each colony, a beetle blast trap was placed in each hive box. The traps were collected and replaced at time intervals and the number of beetle caught in the traps was recorded during the 73-day period of the experiments.

Data Analysis

The number of adult SHB collected was analyzed using a repeated measures analysis (PROC Mixed; SAS Institute 1996). Treatments were modeled as fixed effects; date, and date-by-treatment interactions were modeled as random effects. The data on numbers of SHB collected per hive were transformed to log (x + 1) to satisfy the assumptions of normality before analysis.

Mortality data of SHB in the field trials were subjected to Probit analysis to generate dose-mortality regression lines, the LC50, LC90 and LT values using POLO program. Dose-mortality responses were corrected for control mortality by the POLO program. Virulence among fugal isolates were considered significantly different if the 95% confidence limit (CL) at the LC50 with no overlap.

- Data Collection
- PhotoGrid_1349475923562.jpg

Research results and discussion:

The populations of SHB collected in the traps varied significantly over the course of the experiments. Data indicated that SHB populations in bee hives treated with Metarhizium 5680 exhibited two buildups; the first increase was at day 17 which was followed by the second one at day 59. Mortality of bee hives under the Metarhizium 5680 spores occurred on day 45 and reached 100% at day 73. Beetle infestations in colonies treated with fungal spores produced by Envera showed a peak in SHB infestation levels on day 31 and day 59 with a total count of 53 SHBs and 42 SHBs, respectively. Mortality of bee hives treated with Metarhizium 3020 spores also began at day 45, but reached only 40% at the end of the experiments on day 73. Similar patterns of SHB populations were found in control colonies. In these bee hives, beetle population buildups occurred at day 31 and day 59 with a total count of 18 SHBs and 63 SHBs, respectively. Unlike both bee hives treated with fungal spores, levels of SHB infestation in the control treatment increased from day 59 to day 73. Hive death in the control group occurred at day 45 and reached 90% mortality at day 73. The numbers of SHB infestations were significantly different between treatments 73 days after the first applications ($F = 2.73$; $df = 2, 24$; $P = 0.0053$). The time effect (a measure of within-treatment variability over time) was statistically significant ($F = 6.23$; $df = 5$; $P = 0.0016$). Small hive beetle infestations

were significantly reduced in fungal treated colonies compared to the controls at the end of the 73-day experiment period.
Mortality of bee hives was significantly different between treatments at the end of the experimental period (F = 0.23; df = 2, 22; P = 0.023). The time effect was significant (F = 4.6; df = 5, P = 0.025). Colonies treated with Metarhizium 5680 spores exhibited significantly low hive mortality at the end of the experiments. Both of the fungal spore treatments displayed a decrease in SHB infestation levels greater than the control from day 1 to day 73 suggesting that both of the fungal spore treatments provided a various degree of control of SHB populations during the course of the experiments. The 2g of fungal spores of Metarhizium 5680 used in the experiment were the most efficacious in controlling SHB populations while maintaining colony health throughout this field trial. The 10g of Metarhizium 3020 spores also reduced SHB populations; however, colony health was not maintained. Mortality of bee hive was found in all treatment groups. Although, SHB populations were reduced in hives treated with Metarhizium 3020 spores, hive mortality was the highest in this treatment group, suggesting other factors influence the health of the colonies. These factors may include nutrition, temperature, and initial SHB densities. This experiment was conducted twice in two field locations however the previous trials produced poor results. The first experiment was conducted at an apiary in Wewahitchka, FL. Fully established honey bee colonies from an apiary were used to simulate conditions in beekeeping operations. Three groups of twenty established colonies with heavy infestations of small hive beetles distributed in three rows were used for the experiment. Each colony was 3m apart from the next colony and each row was 15m apart. Each of the colonies was randomly assigned a treatment. In treatment 1, the base pan of the hives was treated with 4 lbs. of sand mixed with 55ml of M. anisopliae, water, and 1% triton solution and a beetle trap. Treatment 2 contained beetle blaster traps with untreated sand, and treatment 3 served as controls with untreated sand only (60 hives total). To estimate the number of beetles in each colony at the end of 28 day experimental period, all traps were replaced twice (day 7 and day 14). The results of these field trials showed that this application method did not effectively control SHB populations.

- Fungi recovered from beetle blaster trap
- SHB larvae in soil treatments

Participation Summary

Educational & Outreach Activities

PARTICIPATION SUMMARY:

Education/outreach description:

Wheeler, S. (2012). The susceptibility of the small hive beetle (Aethina tumida Murray) to fungal pathogens and new generations of insecticides. Master's Thesis, Florida Agricultural and Mechanical University, Tallahassee, FL.

Project Outcomes

Project outcomes:

Control of the small hive beetle populations using fungal pathogens will be cost effective, environmentally friendly and safe for honey consumption.

Farmer Adoption

Our research was conducted with honeybee colonies provided by a local beekeeper. The field trials indicated that there was a significant decrease in small hive beetle populations treated with Metarhizium. As a result of our research other local beekeepers have expressed interest in participating in our field trials. One beekeeper has adopted our soil treatment technique so far; the results of these field trials indicated that our protocol could benefit other beekeepers.

Recommendations:

Areas needing additional study

The time of application of the microbial control agents, the dose and frequency of applications of the fungal pathogens will need additional investigations.