BIO-CONTROL POTENTIAL OF THE ENTOMOPATHOGENIC FUNGUS BEAUVERIA BASSIANA FOR CONTROLLING MOLE CRICKETS, GRYLLOTALPA GRYLLOTALPA (ORTHOPTERA: GRYLLOTALPIDAE) Ahmed Abdu Hamed Amin Idris Salam Abdel-wahab Plant Protection Res. Institute, ARC Giza, Egypt

Abstract

Mole crickets are one of the most destructive groups of vegetable, field crop, and turf and pasture grass pests in Egypt and worldwide based on the damage they cause and the high cost of control. The use of conventional insecticides in field crops and vegetable fields often exposes environmentally sensitive areas such as irrigation lakes, residential homes, and wildlife to the possible deleterious effects of chemicals. A more environmentally friendly control agent is the entomopathogenic soil fungus, *Beauveria bassiana*. The present work aims to evaluate the efficacy of using the biological agent *B. bassiana* in controlling the mole cricket, *Gryllotalpa* sp. Field studies were conducted during 2007 and 2008 to study and evaluate the biologically based management program of key cotton pests in Bani-Sweif Governorate, located 150 km south of Cairo, Middle Egypt Region. Results showed that the total number of the dead insects after seven days was 509 in the treatment of Hostathion - H 40 % in 2007 and 334 in 2008 followed by Biosect (6.4 X10¹⁰), Biosect (4.8 X10¹⁰), Biosect (4 X10¹⁰) and Biosect (3.2X X10¹⁰), respectively, in both seasons. Also, the area of new surface tunnels produced was significant with mean values for each set of replications varying from a low of 26.608 cm² in the Biosect (6.4 X10¹⁰) treatments in 2008 to a high of 281.869 cm² in the control treatments in 2007.

Introduction

Mole crickets are one of the most destructive groups of vegetable, field crop, and turf and pasture grass pests in Egypt and worldwide based on the damage they cause and the high cost of control. The root feeding nature and production of subterranean tunnels by these hemimetabolous insects results in desiccation and destruction of the root zone, which causes plant stress or loss of yield quality. Additionally, acoustic-calling chambers built by the males in the spring and early summer months appear as large holes on the surface of the ground.

The use of chemicals for control of mole crickets is the most common strategy used by vegetable, field crop and golf course superintendents and turf grass managers. Although many different chemicals have proven moderately successful in reducing mole cricket damage, they cannot be employed without concerns over the costs and potential environmental impacts. Due to the soil habitat of mole crickets, higher doses and repeated applications of treatments are often needed to achieve acceptable levels of control. The impacts of chemicals on groundwater quality and non-target wildlife, as well as the concerns over human exposure and persistence and degradation in the environment have resulted in the search for alternative methods of control. Natural enemies from the regions where mole crickets originated have been imported to serve as biological control agents, but difficulties in establishing sufficient populations and over wintering have limited their success. Other potential biological control agents include entomogenous nematodes, as well as fungi, both of which have shown potential for safe management.

Developing IPM strategies requires reliable information. Three main areas are:

- Knowledge concerning all normal inputs required for growing the turf not only what they are but also why they are required. This is supplemented by knowing pest life cycles and which management practices disrupt or influence them to reduce pest numbers.
- Understanding the logic behind a management practice, rather than just doing it "because that is the way we have always done it," allows the homeowner to make decisions to alter these practices to reduce pest problems or encourage turf growth to overcome or tolerate the pest.

• Use of a monitoring system to carefully follow pest trends to determine if a pesticide will be necessary and, if so, when it would be most effectively applied. Ideally, monitoring systems are based on known economical or aesthetic threshold levels. Unfortunately, in many cases, these thresholds are not specifically known, thus are determined to reflect local conditions and threshold levels tolerated by clientele

Although there are over 70 species of mole crickets worldwide (Otte 1994), only a few are found in Egypt. *Gryllotalpa africana* Palisot de Beauvois and *G. gryllotalpa* L. are significant pests at this time. *Gryllotalpa africana*, the African mole cricket, has a long history of interceptions by PHIS-PPQ since 1961 (Nickle and Castner 1984), .

Mole crickets are hemimetabolous insects that spend their entire life cycle in subterranean tunnels. When mole crickets first appeared and established themselves as pests in the U.S., calcium arsenate and calcium cyanide baits were the most commonly used control measures (Frank and Parkman 1999). When the more effective chemical, chlordane, was discovered in the 1940s, it replaced these other baits and was used for mole cricket control in vegetable crops, turf, and pastures until it was subsequently banned in the 1970s (Frank and Parkman 1999). At that point, chlordane was primarily replaced with organophosphate and carbamate insecticides, which were widely applied for control of both adult and nymphal mole crickets. However, since the passing of the Food Quality Protection Act in 1996, the EPA has begun gradually restricting and deregulating the use of these chemicals (Frank and Parkman 1999). Currently, new classes of chemicals have provided alternative insecticides such as imidacloprid and fipronil for suppression of mole cricket populations. Regardless of the agent, most experts agree that an understanding of natural history is critical for optimal control (Brandenburg 1997, Potter 1998).

Today, the most effective mole cricket control program advocates establishing a complete plan that includes mapping adult activity, monitoring egg hatch, timing the application of pesticides, and providing any necessary follow-up (Brandenburg 1997, Potter 1998). Mapping adult activity in early spring is essential (Cobb and Lewis 1990) because mole crickets often lay their eggs in the same area where they develop, unless significant changes in turf quality or management practices occur (Potter 1998). Additionally, infestations are usually not uniform (Brandenburg 1997), meaning that field-to-field treatment is often unnecessary. Occasionally, damage from adults can be so severe, that treatment is required in the spring (Brandenburg 1997).

To determine areas of high activity, visual inspection of the seedlings should be made, looking for new surface tunnels or acoustic chambers and records should be kept to serve as a guide for locating future egg hatch (Brandenburg 1997). At the same time that activity is being mapped, flights can be monitored with acoustic sound traps (Ulagaraj and Walker 1975, Walker 1982). These traps help keep track of first, peak, and declining flights, indicating when egg hatch is likely to occur. Ngo and Beck (1982) have determined that the calling traps are effective for monitoring flight activity but are not accurate in estimating the actual population.

The need for alternate methods of insect control is evident as public concern over the use of chemicals continues to increase. The use of conventional insecticides in field crops and vegetable fields often exposes environmentally sensitive areas such as irrigation lakes, residential homes, and wildlife to the possible deleterious effects of chemicals. A more environmentally friendly control agent is the entomopathogenic soil fungus, *Beauveria bassiana* (Balsamo) Vuillemin. The success of *B. bassiana* as a pathogen of mole crickets depends on several key factors. The research conducted in the preceding sections addresses many of these factors, which ultimately determine the fate of this fungus as a potential biological control agent for mole crickets. The three key issues examined were the evaluation of the efficacy of various rates and strains, a measurement of the viability of the conidia when applied to fields, and the determination of the ability of the mole crickets to detect and avoid contact with the conidia. The present work aims to evaluate the efficacy of using the biological agent *B. bassiana* in controlling the mole cricket, *Gryllotalpa* sp.

Materials and Methods

Bani Sweif Governorate is located 150 km south of Cairo, Middle Egypt Region, on latitude 29° 25", longitude 31° 30". Two locations were chosen to conduct the field experiments: Wasta District (2007) and Bani Sweif District (2008). Tests were undertaken in areas where the mole cricket is known to occur.

Field studies were conducted during 2007 and 2008 to study and evaluate the biologically based management program of key cotton pests. The experimental area was approximately 4 feddans (16,800 m^2) of cotton (Giza, 90) during the cotton seasons. The area was divided into 4 blocks each approximately 1 feddan (4200 m^2), each block was divided into 6 plots (each plot was 25 X 25 m).



Biological agents used

Beauveria bassiana is a naturally occurring fungal disease that affects a very wide range of insects, including aphids, whiteflies, psyllids, billbugs and caterpillars. Environmental conditions, particularly humidity, seem critical for the applied spores to successfully germinate and infect insects. Newly infected insects often are somewhat light brown. When the fungus sporulates, it covers the insect with white spores.

The commercial product Biosect, containing 32-million spores/cm³ of the *B. bassiana*, was used in four rates: 1, 1.25, 1.5 and 2 liters/feddan. The other treatment used in these studies for comparison was the commercially available cotton insecticide, Hostathion - H 40 % EC (1.25 liter/feddan) and another free of treatments for control. Observations were done 24, 48, 72 hours 5 and 7 days after treatments. Experiments were done using randomized complete blocks.





Treatments

Because granular insecticides are more effective than foliar insecticides, the tested materials were used as poisoned baits. Baits have proven somewhat effective against adults in the fall and spring. They should be applied to moist, not soggy soil, and should not be watered in. Any water, either irrigation or rainfall, immediately after the application of bait diminishes its effectiveness.

Poisoned baits are made by mixing 20 kg of moistened corn bran with the recommended dose of insecticide as well as one kg of molasses. These can be placed along plant seedlings or drier areas of the field to kill night-foraging mole crickets. Soybean oil (1 kg) was used as a protectant base to prevent the bait from sun effects and drying out.

To maximization the benefits from the baits, the soil was moisten by quick irrigation and quick draining (not spongy) that forced the underground insects to surface as well as prepare a humid environment for the fungi.

Sampling

Treatments were distributed in randomized complete blocks design, each block (4 blocks or replicates) contains 6 plots, and each plot has a surface area of 25 X 25 m ($625m^2$). For sampling the dead insects, 5 samples from each plot were taken, with each sample being an approximately 42 m² area of 6 X 7 meters.

After 24 hours, observations of tunneling were made, and new surface tunnels were measured. Each plot was checked for the presence of new surface tunneling after five days, whether the tunnels were vertical or horizontal tunnels or a combination of both, and if tunneling occurred around the perimeter of the plot ("edging"). Additionally, the location and status (alive or dead) of each cricket was determined. Any obvious disruption to the smooth soil surface was categorized as new surface tunneling. Vertical tunnels appeared as small holes descending into the soil profile, while horizontal tunnels looked like trenches on the surface of the soil. The area of new surface tunneling was measured by running string along the length and width of the tunnels and measuring the length of the string pieces. Observations were continued for 48, 72 hours, 5 and 7 days and measurements of the tunnels were done after 5 days.

Statistical Analysis

For all treatments, the mean surface area of tunneling, as well as the percentage occurrence of each behavior, were analyzed by factorial analysis of variance using the General Linear Models Procedure and Least Square Means of SAS (PROC GLM, SAS Institute 2001). T-tests ($P \le 0.5$) were used to calculate the least significant differences (LSD) in surface area means and mean percentage occurrences of each tunneling feature.

Results & Discussion

Although all tests were not performed simultaneously, each trial included its own set of controls. The mean area of surface tunnels and cumulative percentage occurrence of each tunneling behavior for control treatment only were compared by LSD to determine if significant differences occurred in the controls between years or between trials within years. Figures 1 and 2 demonstrate the efficacy of the different concentrations of Biosect as well as Hostathion - H 40 % EC (1.25 liter/feddan) for comparison on the mole cricket *G. gryllotalpa* in Bani Sweif Governorate, Middle Egypt. As it can be seen from the figures the total number of the dead insects after seven days was 509 in the treatment of Hostathion - H 40 % in 2007 and 334 in 2008 followed by Biosect (6.4×10^{10}), Biosect (4.8×10^{10}), Biosect (3.2×10^{10}), respectively, in both seasons.



Figure 1. Efficacy of the different concentrations of Biosect on the mole cricket *Gryllotalpa gryllotalpa* in Bani Sweif Governorate, 2007 cotton growing season.



Figure 2. Efficacy of the different concentrations of Biosect on the mole cricket *Gryllotalpa gryllotalpa* in Bani Sweif Governorate, 2008 cotton growing season.

New surface tunneling

The presence of new surface tunneling verified that the cricket had passed through and come in contact with the treatment layer on the original soil surface. The area of the new surface tunnels produced was significant (F = 3.76; P = 0.0016), with mean values for each set of replications varying from a low of 26.608 cm² in the Biosect (6.4 X10¹⁰) treatments in 2008 to a high of 281.869 cm² in the control treatments in 2007 tests (Figure 3).

In the *B. bassiana* treatments, death was not expected at 24 or 48 hours post treatment since death by beauveriosis usually occurs 3-4 days after attachment of conidia to the insect cuticle (Inglis et al. 1997). It was therefore not surprising that there was very little mortality observed in any treatment. Based on the findings of new surface tunneling, it does not appear that the crickets were able to detect any of the treatments to a degree strong enough to deter surface tunneling completely as observed by Villani et al. (2002). Due to the significant differences in mean area of surface tunneling, it appears that the tunneling activity of the mole crickets was minimized by the presence of Biosect (6.4 X 10^{10}), and Biosect (4.8 X 10^{10}): 26.608 cm² and 47.909 cm², respectively, in 2008.

The method of application can improve contact of the pathogen with the insect pests targeted. Application methods for some insect pests have been devised. Methods for subterranean insect pests (such as mole crickets) still need development because broadcast application on the soil surface is likely to be prohibitively expensive as well as leading to the rapid demise of the pathogen when it encounters bright sunlight. Alternative methods are injection into the soil, or incorporation of the pathogen into an attractant such as bait. Materials used to make the bait obviously are important, because it must be very attractive to the target pest while preserving the pathogen.

Each of these fungal pathogens has been collected from various insect species from various places. Capabilities of these various collections of pathogens, called strains, differ. Under experimental comparison using *G. gryllotalpa* in the laboratory, some strains have been found to kill all mole crickets they contacted, whereas others killed a much smaller proportion. Code numbers and letters

have designated many strains of *B. bassiana*; they are not distinct species. Speed of kill by these various strains also differs, though in general all are slower than chemical pesticides now in use.

Development of a method of applying fungal pathogens against *G. gryllotalpa* nymphs is a worthwhile project because biological control agents now in use have little or no effect against nymphs. If successful, it could replace use of chemical pesticides now used against the nymphs, with greater safety to non-target organisms.













Figure 3. Mean area (cm²) of surface tunnels produced after 72 hours by adult mole crickets in response to different treatments in Bani Sweif Governorate, 2007 & 2008 cotton growing seasons.

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