

ABSTRACT

THOMPSON, SARAH RACHEL. Enhancing the Efficacy of *Beauveria bassiana* for Mole Cricket (Orthoptera: Gryllotalpidae) Control in Turfgrass. (Under the direction of Rick L. Brandenburg.)

The objectives of this research conducted with the entomogenous fungus, *Beauveria bassiana*, include: a) to measure the effect of UV exposure and applied irrigation on the viability of conidia when applied to turfgrass b) to determine the type of interaction that exists between various strains of the fungus and other insecticides (imidacloprid and diatomaceous earth) and c) to elucidate the mechanism that mole crickets use to detect and avoid contact with the conidia. All three studies focused on two strains of *B. bassiana*, 5977 and 3622, that were originally isolated from orthopteran hosts and obtained from the ARS Collection of Fungal Cultures (Ithaca, NY). Other strains evaluated include strains DB-2 and MC, which were isolated from a darkling beetle and a tawny mole cricket, respectively. These four strains were propagated and formulated by JABB of the Carolinas, Inc. (Pine Level, NC). A commercially available strain, GHA formulated as BotaniGard[®] ES, from Emerald Bioagriculture Corporation (Butte, MT) was also evaluated.

Field research to evaluate the impact of UV light and moisture on conidial viability was conducted on Bermudagrass *Cynodon dactylon* (L.) plots at BASF Research Station (Fuquay-Varina,, NC) in July-August 2004 and August-September 2005. In a baseline viability study, there were no differences between strains 5977 and 3622 and both strains were able to persist in the environment up to 21 days after application. There were significant differences overall for both strains at two different levels of irrigation, with plots receiving 15.3 cm of irrigation during the study maintaining spore viability better than plots receiving 7.5 cm of irrigation. Different carrier formulations also significantly affected

conidial viability for strain 3622. Oil formulations containing an optical brightener or magnesium silicate clay increased conidial viability by approximately 10% compared to the oil or clay alone formulations.

Laboratory topical bioassays were conducted in June 2004 and 2005 on adult southern mole crickets to determine the interaction between *B. bassiana* and diatomaceous earth (DE) or imidacloprid. For all three strains tested, 5977, 3622, and GHA, there was significantly more mortality in combination treatments involving the fungus and DE compared to either treatment alone at a sublethal dose. These interactions were synergistic for strains 5977 and 3622. The percentage mortalities caused by combination treatments involving sublethal doses of imidacloprid and *B. bassiana* were less than additive for all three strains. This interaction was antagonistic for strain 5977. Combination treatments with diatomaceous earth caused as much mortality as high rate doses of the fungus alone for all three strains.

Behavioral studies using tawny nymphs and southern adult mole crickets were conducted during the spring and fall of 2005 to determine the mechanism that mole crickets use to detect and avoid contact with *B. bassiana* conidia. A two-chamber test unit was designed utilizing infrared emitters and detectors to monitor mole cricket movement in areas adjacent to and containing fungal treated or untreated sand. Significant variations in behavior that suggest a chemosensory mechanism of detection were observed between treated and untreated chambers. Avoidance behaviors were more extreme for the southern mole crickets, suggesting a more sensitive response. Results from these studies emphasize the impact of abiotic factors on the use of *B. bassiana* as a biological control agent for mole crickets and suggest methods to increase efficacy of the fungus.

**ENHANCING THE EFFICACY OF *BEAUVERIA BASSIANA* FOR MOLE
CRICKET (ORTHOPTERA: GRYLLOTALPIDAE) CONTROL IN TURFGRASS**

by
SARAH RACHEL THOMPSON

A dissertation submitted to the Graduate Faculty of
North Carolina State University
in partial fulfillment of the
requirements for the Degree of
Doctor of Philosophy

ENTOMOLOGY

Raleigh

2006

APPROVED BY:

Chairman of Advisory Committee

BIOGRAPHY

Sarah Rachel (Devereux) Thompson was born the fourth of six children on 18 August 1977 in Baltimore, Maryland. She received her elementary and secondary education in Parkton, Maryland, graduating from Hereford High School in 1995.

Sarah attended University of Maryland in College Park, graduating in 1999 with a Bachelor of Science degree in Secondary Science Education with an emphasis in Biology. After completing this degree, she taught sixth grade general science at Eagles Landing Middle School in Boca Raton, Florida for two years. The author began a Master of Science program in July 2001 under the supervision of Dr. Rick L. Brandenburg in the Department of Entomology at North Carolina State University. Her thesis research was an examination of mole cricket behaviors and other factors that affect the success of biological control agents. She completed her M.S. in December 2003, and since that time, has been exploring methods to enhance the efficacy of an entomopathogenic fungus for control of mole crickets in turfgrass. This dissertation research project has also been conducted under the supervision of Rick Brandenburg.

On 31 August 2002, she married Bryan Keith Thompson in Baltimore. They reside in Raleigh with their two dogs, Max and Jake.

ACKNOWLEDGMENTS

The Center for Turfgrass Environmental Research and Education in Raleigh, NC provided generous financial support for this research. I am indebted to this organization and the Department of Entomology at North Carolina State University for giving me the chance to complete both of my graduate degrees.

I thank Dr. Rick Brandenburg for all the opportunities he has given me during my time working with him for both my M.S. and Ph.D. degrees. These opportunities have helped me to develop into the well-rounded person I have always aimed to be. He is a truly great mentor who lives his life the way I hope I someday can live mine, committed equally to his professional and personal lives. He has always treated me as an equal and with utmost respect. He has also always made himself available to me when I need advice or a friend to talk to and for all these things I am so grateful. I can't thank him enough for the interest he has taken in me as a person, rather than just a student.

Drs. Jim Arends, Wayne Brooks, and Wes Watson have provided helpful advice, expertise, and humor and I am so appreciative for having them as members of my committee. Their critique of this dissertation was invaluable. I am especially indebted to Drs. Arends and Brooks who have served on both my M.S. and Ph.D. committees, an effort that has streamlined this entire process for me.

In Dr. Brandenburg's lab, I would like to acknowledge Brian Royals, Brenda Watson, and Dr. Peter Hertl for their assistance and support over the past four and a half years. From the

Department of Statistics, I thank Dr. Cavell Brownie for her patience and help with the statistical analysis of my data. In the Department of Biological and Agricultural Engineering, many thanks to Dr. Gary Roberson, Barry Lineberger, and Phil Harris. I have truly enjoyed working with all three of these individuals whose expertise has been instrumental in the behavioral studies.

Without the cooperation and assistance of Steve White at The Lakes Country Club (Boiling Springs Lakes, NC), Brent at Sandpiper Bay Golf Course (Sunset Beach, NC), and David Mann at BASF Research Station (Fuquay-Varina, NC), my research would have not been possible. A special thanks to Bill Black at JABB of the Carolinas for his friendship and spiritual guidance over these years.

To all of my family...you are my constant source of inspiration. Thank you for understanding and not resenting me for the decisions that I have made that have taken me away from you. I think of you all every day and love you with all of my heart. Mom, without your unwavering belief in me, I don't know where I would be today. Today and always, thank you for everything.

And, most importantly, to my husband, Bryan- there is no doubt in my mind that I would not have been able to accomplish this without you in my life. You have been there for me every step of the way. For all the things that you have done for me and with me, thank you. Not a day goes by that I don't count my blessings and wonder what I have done to deserve you as my partner in this life. I love you.

TABLE OF CONTENTS

	Page
LIST OF TABLES.....	ix
LIST OF FIGURES.....	x
INTRODUCTION.....	1
IMPACT OF MOISTURE AND UV DEGRADATION ON <i>BEAUVERIA BASSIANA</i> (BALSAMO) VUILLEMIN CONIDIAL VIABILITY IN TURFGRASS.....	3
Abstract.....	4
Introduction.....	5
Materials and Methods.....	7
Propagation of Fungal Isolates.....	7
Application of Fungal Conidia to Turfgrass.....	8
Conidial Suspension Preparation.....	10
Viability Assessment using Fluorescence Microscopy.....	11
Statistical Analysis.....	12
Results.....	12
Baseline Study Conidial Viability.....	12
Irrigation Study Conidial Viability.....	14
UV Protectants Study Conidial Viability.....	15
Weather Data.....	16
Discussion.....	17
References.....	21

EFFECT OF COMBINING IMIDACLOPRID AND DIATOMACEOUS EARTH WITH

BEAUVERIA BASSIANA (BALSAMO) VUILLEMIN ON MOLE CRICKET

(ORTHOPTERA: GRYLLOTALPIDAE) MORTALITY.....31

Abstract.....32

Introduction.....33

Materials and Methods.....36

Propagation of Fungal Isolates.....36

Collection of Mole Crickets.....36

Pre-treatment of Substrate.....37

Treatment of Mole Crickets with Fungus.....38

Incubation of Cadavers.....39

Statistical Analysis.....40

Results 40

Discussion.....43

References Cited.....47

A TECHNIQUE TO MONITOR INSECT BEHAVIOR UTILIZING INFRARED

EMITTERS AND DETECTORS.....56

Treatment Containers.....57

Photosensors.....58

Software.....59

Summary.....61

References Cited.....62

MECHANISM OF ENTOMOPATHOGENIC FUNGI DETECTION AND AVOIDANCE BY MOLE CRICKETS (ORTHOPTERA: GRYLLOTALPIDAE).....	72
Abstract.....	73
Introduction.....	74
Materials and Methods.....	76
Propagation of Fungal Isolates.....	76
Collection of Mole Crickets.....	77
Behavioral Studies.....	78
Trial I.....	80
Trial II.....	80
Statistical Analysis.....	82
Results and Discussion.....	82
First Photosensor Trigger.....	82
Number of Photosensor Triggers.....	84
Presence of Tunneling.....	85
Final Location of Cricket.....	86
References Cited.....	90
CONCLUSIONS.....	99
APPENDICES.....	102
EFFICACY OF HIGH AND LOW RATES OF <i>BEAUVERIA BASSIANA</i> FOR CONTROL OF MOLE CRICKET NYMPHS IN TURF, 2004.....	103
EFFICACY OF SINGLE AND MULTIPLE APPLICATIONS OF <i>BEAUVERIA</i>	

<i>BASSIANA</i> FOR CONTROL OF MOLE CRICKET NYMPHS IN TURF, 2005.....	108
--	-----

LIST OF TABLES

EFFECT OF COMBINING IMIDACLOPRID AND DIATOMACEOUS EARTH WITH *BEAUVERIA BASSIANA* (BALSAMO) VUILLEMIN ON MOLE CRICKET (ORTHOPTERA: GRYLLOTALPIDAE) MORTALITY:

1. Mean mole cricket survival time (days) for 2004 and 2005 topical bioassays and mean survival time averaged over both years.....51
2. Statistical values for topical bioassay synergy tests (day 28 mortality) involving *B. bassiana* and diatomaceous earth or imidacloprid (N=8).....52

EFFICACY OF HIGH AND LOW RATES OF *BEAUVERIA BASSIANA* FOR CONTROL OF MOLE CRICKET NYMPHS IN TURF, 2004:

1. Mean mole cricket damage ratings.....106
2. Materials tested for arthropod management.....107

EFFICACY OF SINGLE AND MULTIPLE APPLICATIONS OF *BEAUVERIA BASSIANA* FOR CONTROL OF MOLE CRICKET NYMPHS IN TURF, 2005:

1. Mean mole cricket damage ratings.....111
2. Materials tested for arthropod management.....112

LIST OF FIGURES

IMPACT OF MOISTURE AND UV DEGRADATION ON *BEAUVERIA BASSIANA* (BALSAMO) VUILLEMIN CONIDIAL VIABILITY IN TURFGRASS:

1. Mean percentage conidial mortality for *B. bassiana* strains 5977 and 3622 over time (BASF field research station, Fuquay-Varina, NC).....25
2. Mean percentage conidial viability for *B. bassiana* strains 5977 and 3622 at two different irrigation regimes (high and low) over time (BASF field research station, Fuquay-Varina, NC).....26
3. Mean percentage conidial viability for four different UV protectant formulations of *B. bassiana* strain 3622 over time (BASF field research station, Fuquay-Varina, NC).....27
4. Mean daily air temperature (°C) during 2004 and 2005 field studies, indicating start of baseline, irrigation, and UV protectant viability tests. 2004 data include temperatures from 5 Jul to 11 Aug; 2005 data include temperatures from 8 Aug to 15 Sep.....28
5. Mean daily soil temperature (°C) at depth of 5 cm during 2004 and 2005 field studies, indicating start of baseline, irrigation, and UV protectant viability tests. 2004 data include temperatures from 5 Jul to 11 Aug; 2005 data include temperatures from 8 Aug to 15 Sep.....29
6. Daily rainfall amounts (cm) during 2004 and 2005 field studies, indicating start and end of irrigation viability test. 2004 data include temperatures from 5 Jul to 11 Aug; 2005 data include temperatures from 8 Aug to 15 Sep.....30

EFFECT OF COMBINING IMIDACLOPRID AND DIATOMACEOUS EARTH WITH *BEAUVERIA BASSIANA* (BALSAMO) VUILLEMIN ON MOLE CRICKET (ORTHOPTERA: GRYLLOTALPIDAE) MORTALITY:

1.	Mean percentage mole cricket mortality at 28 DAT for <i>B. bassiana</i> strain 5977 treatments. Means followed by the same letter are not significantly different (LSD t-test, $P = 0.05$, SAS Institute, 2003).....	53
2.	Mean percentage mole cricket mortality at 28 DAT for <i>B. bassiana</i> strain 3622 treatments. Means followed by the same letter are not significantly different (LSD t-test, $P = 0.05$, SAS Institute, 2003).....	54
3.	Mean percentage mole cricket mortality at 28 DAT for <i>B. bassiana</i> strain GHA treatments. Means followed by the same letter are not significantly different (LSD t-test, $P = 0.05$, SAS Institute, 2003).....	55

A TECHNIQUE TO MONITOR INSECT BEHAVIOR UTILIZING INFRARED EMITTERS AND DETECTORS:

1.	Sample behavioral test unit, showing entrance tube (A), “T” connector (B), and photosensors (C).....	64
2.	Sensor circuit illustrating supply voltage, infrared emitter and detector, resistors, and signal output.....	65
3.	Bug VI front panel (i.e. user interface) showing LED indicators for each event, control buttons for Bugs #1-12, start and elapsed times for each bug, and stop and reset buttons.....	66
4.	Bug VI wiring diagram.....	67
5.	Main “while” loop.....	68
6.	Secondary “while” loop.....	69

7.	Case structure that writes data to file when sensor state changes.....	70
8.	Example of data spreadsheet that shows the time associated with each occurrence of a sensor being blocked (1) and then unblocked (-1).....	71

MECHANISM OF ENTOMOPATHOGENIC FUNGI DETECTION AND AVOIDANCE BY MOLE CRICKETS (ORTHOPTERA: GRYLLOTALPIDAE):

1.	Sample behavioral test unit, showing entrance tube (A), “T” connector (B), and photosensors (C). Note the extensive tunneling in the left (untreated) container in comparison to the right (treated) container.....	94
2.	Percentage occurrence of mole cricket triggering <i>B. bassiana</i> -treatment photosensor first.....	95
3.	Percentage of total photosensor triggers that occurred at <i>B. bassiana</i> -treated container.....	96
4.	Percentage occurrence of tunneling in <i>B. bassiana</i> -treated container; * indicates treatment differs significantly from control-control treatment (Likelihood Ratio Chi ² Pairwise Comparison Test, $P \leq 0.05$, SAS Institute, 2003).....	97
5.	Percentage occurrence of mole cricket choosing to stay in <i>B. bassiana</i> -treated container; * indicates <i>B. bassiana</i> treatment differs significantly from control-control treatment (Likelihood Ratio Chi ² Pairwise Comparison Test, $P \leq 0.05$, SAS Institute, 2003).....	98

INTRODUCTION

Mole crickets are serious pests of turfgrass in southeastern U.S. due to the substantial damage they cause and the high cost of control. The two major pest species, the tawny mole cricket *Scapteriscus vicinus* Scudder and the southern mole cricket *Scapteriscus borellii* Giglio-Tos, both produce extensive underground tunneling which results in desiccation and destruction of the turf root zone. While southern mole crickets are predominantly predatory, tawny mole crickets feed on the roots of the grass, which makes them the more significant pest of the two species. In the springtime, the acoustic calling chambers produced by the male crickets to attract females for mating are problematic since they appear as large holes or mounds on the surface of the ground. Severe infestations can lead to total turf loss and invasion of opportunistic weeds. The damage produced by mole crickets is unacceptable in turfgrass, a commodity whose value is based almost entirely on aesthetics.

Control of mole crickets in managed turfgrass is usually accomplished through the use of chemical insecticides. Due to the soil habitat of these pests, high rates and repeated applications are often necessary to achieve acceptable levels of control. A large proportion of turfgrass is in close proximity to environmentally-sensitive areas, such as irrigation lakes, residences, or wildlife habitats. As a result, there is some concern over the use of synthetic insecticides in these areas. There is additional concern over the impact that pesticides may have on groundwater sources and the potential for long-term persistence in the environment.

Negative press and public perception of conventional pesticides has resulted in a regulatory movement in Canada and some areas of the U.S. to ban the use of these products when they are applied solely for cosmetic enhancement, as is the case in turfgrass. It has therefore been necessary for research programs to explore alternative methods of control.

Biological control agents, which include predators, parasites, and pathogens, are generally thought to offer safer options for management. Natural enemies have been imported into the U.S. from the native regions of mole crickets, but difficulty in establishing populations and limited overwintering have limited their success for control. Other potential biological control agents for these destructive turfgrass pests include entomopathogenic nematodes and fungi.

Beauveria bassiana (Balsamo) Vuillemin, a naturally-occurring soil fungus, has been shown to be pathogenic to hundreds of different species of insects. There are many notable success stories with this fungus including control of grasshoppers, houseflies, and darkling beetles. Some factors that determine the success of any entomopathogenic fungus include adequate contact between the host and conidia, strain-specific pathogenicity towards the targeted pest, and the ability of the conidia to maintain viability in the field. Our previous research explored these factors to determine if *B. bassiana* has potential for environmentally-safe control of mole crickets. Results indicated that conidia were capable of maintaining viability in turfgrass for up to one month after application, pathogenicity varied with Orthoptera isolates killing the most crickets in the shortest time, and strain-specific avoidance behaviors were evoked in the crickets. This project has focused on ways to increase the efficacy of orthopteran strains of *B. bassiana* for control of mole crickets. The objectives of our research were: a) to measure the effect of moisture and UV exposure on conidial viability in the field b) to determine the type of interaction (additive, synergistic, or antagonistic) that exist between the fungus and other insecticides and c) to elucidate the mechanism employed by mole crickets to detect and avoid contact with the conidia.

Biological Control

Thompson et al.: Effects of moisture and UV light on *B. bassiana*

**Impact of moisture and UV degradation on *Beauveria bassiana* (Balsamo) Vuillemin
conidial viability in turfgrass**

SARAH R. THOMPSON^{1a}, RICK L. BRANDENBURG¹, AND JIM J. ARENDS²

¹ Department of Entomology, North Carolina State University, Box 7613, Raleigh, NC
27695

² JABB of the Carolinas, PO Box 310, Pine Level, NC 27568

^a Corresponding author: Phone: (919) 513-2209 Fax: (919) 515-7746

E-mail: sarah_thompson@ncsu.edu

Abstract

Beauveria bassiana conidial viability in turfgrass was evaluated using a two-component nucleic acid stain and fluorescence microscopy. Turfgrass samples along with the top 5 cm of soil were used for conidial extraction and viability evaluation on 1, 2, 3, 7, 14 and 21 days after treatment. There were no differences in conidial viability between two Orthoptera strains, 3622 and 5977, and both strains were able to persist in a sandy loam soil for up to three weeks after application. High and low irrigation levels were applied to each of the two strains and results show that higher irrigation (5.1 cm) maintains conidial viability better than a low irrigation level (2.5cm). Mean conidial viability was approximately 8-12% greater in plots with the high irrigation regime. Rather than significantly increase soil moisture levels, it is hypothesized that the greater amount of irrigation helps to move the conidia deeper into the thatch layer and soil profile, an area that provides protection from damaging surface temperatures and UV exposure. Rainfall that occurred during the beginning of the 2005 test minimized the irrigation effect, and the irrigation treatment differences were more pronounced after 48 hours. Four different UV protectants were evaluated for an impact on conidial viability of strain 3622. Two protectants, an optical brightener and magnesium silicate clay, when added to an emulsifiable oil formulation, significantly increased conidial viability on all evaluation dates. The clay particles act as a sunlight blocker while the optical brightener absorbs UV light. The combined protection from the oil and the additive increased conidial viability by approximately 10% on all evaluation dates. Results from this study provide insight into ways to increase entomopathogenic fungal viability under field conditions.

Keywords: *Beauveria bassiana*; conidial viability; fluorescence microscopy; irrigation; optical brightener

1. Introduction

Beauveria bassiana (Balsamo) Vuillemin is one of the most widely researched and promising species of pathogenic fungi used for insect control, virulent to over 200 different species of insects (Feng et al., 1994). The conidia are naturally-occurring in soil, and therefore, are optimal for environmentally-safe control of soil insect pests. Many root-feeding pests of turfgrasses, such as mole crickets (Orthoptera: Gryllotalpidae: *Scapteriscus* spp.), are soil dwelling. The use of conventional insecticides in turfgrass has received a lot of negative attention in recent years, based on the argument that chemicals should not be used to enhance the aesthetics of a plant, but only in cases where there is potential for crop, livestock, or human health loss. Additionally, a large proportion of managed turfgrass is in close proximity to areas where there may be concern over synthetic insecticide usage, such as water sources, residences, or wildlife habitats (Thompson et al., 2005). Research focusing on the development of environmentally-safe control options, such as microbial agents, has been encouraged due to these societal concerns (Goettel et al., 1995).

The use of *B. bassiana* for control of soil pests depends on the ability of the conidia to survive and infect the target host in that environment (Lingg and Donaldson, 1981; Inglis et al., 1997a). The persistence of the conidia in the environment is influenced by many different environmental conditions, including water availability, temperature (Inglis et al., 1996; Inglis et al., 1997b; Hallsworth and Magan, 1999), and UV light (Moore et al., 1993; Morley-Davies et al., 1995; Inglis et al., 1995a). Some studies have attempted to show a correlation between high temperatures and reductions in spore viability (Inglis et al., 1996;

Inglis et al., 1997b; Hallsworth and Magan, 1999) but the technique used to determine viability in these tests was a determination of potential for germination, not actual viability. Lingg and Donaldson (1981) showed that soil moisture content caused fluctuations in conidial half-lives, but again, the evaluation of conidial viability was based on plating the recoverable conidia on a growth medium and evaluating for colony development after a pre-determined amount of time. Other studies that attempted to quantify the effects of soil moisture and temperature on *B. bassiana* persistence also used germination (Krueger et al., 1991) or infectivity (Studdert and Kaya, 1990; Fuxa and Richter, 2004) as measures of conidial viability. A different evaluation technique that utilizes fluorescence microscopy and a two-component nucleic acid stain has shown that *B. bassiana* conidia are able to persist and maintain viability at low levels for up to one month after application to turfgrass (Thompson et al., 2005). Conidia of various fungi, including *Entomophaga maimaiga* Humber, Shimazu, & Soper, *Zoophthora radicans* (Brefeld) Batko (Firstencel et al. 1990), *Paecilomyces fumosoroseus* (Wize) Brown & Smith, and *B. bassiana* (Schading et al. 1995) have been evaluated using this method.

Exposure to the UV-B portion of solar radiation (290-320nm) has a great impact on all living organisms; the negative effects include direct damage to DNA, which can lead to mutations, and the production of highly reactive and deleterious radicals (Ignoffo et al., 1977). Most studies involving UV exposure to entomopathogenic fungi have explored the effects when the conidia are applied to plant leaves or other substances and directly exposed to artificial or natural sources of UV light (Inglis et al. 1995a; Morley-Davies et al., 1995; Edgington et al., 2000). These studies do not accurately represent the amount of UV-light that conidia sprayed on turfgrass may be exposed to. Irrigation is immediately applied after

application of *B. bassiana* conidia to turfgrass (Hertl and Brandenburg, 1998; Xia et al., 2000) and this allows for the movement of spores into the thatch and upper soil profile, where exposure to UV light is minimized, albeit not eliminated. One of the objectives of our research was to evaluate the impact of various UV protectants on *B. bassiana* conidial viability over time in turfgrass.

Most entomopathogenic fungi require a minimum of 95% relative humidity (RH) on the surface of the insect cuticle for germination, penetration, and infection to occur (Hallsworth and Magan, 1999). Some studies have shown that *B. bassiana* conidia can infect host insects in the absence of these RH levels (Ferron, 1977), suggesting that the microenvironment immediately next to the insect cuticle is not representative of the atmospheric RH (Ramoska, 1984). Although high atmospheric RH levels may not be necessary for infection to occur, available water must be freely available to the conidia (Hallsworth and Magan, 1999) before infection in order to prevent desiccation and maintain viability. Based on this premise, the second objective of our research was to evaluate the impact of two different levels of irrigation on *B. bassiana* conidial viability in turfgrass, a commodity wherein irrigation is often applied on a regular basis.

2. Materials and Methods

Propagation of Fungal Isolates

The two strains of *B. bassiana* used in this study, 5977 and 3622, were obtained as cultures from the ARS Collection of Entomopathogenic Fungal Cultures (Ithaca, NY). Strain 5877 is IMI (331275) isolate and its origination is specified as Orthoptera with no specific location. Strain 3622 was isolated by J.L. Stimac in Florida from the tawny mole cricket, *Scapteriscus vicinus*. These strains were chosen since both originate from

orthopteran hosts and previous research indicates that strains isolated from a close relative of the target host are the most pathogenic (Thompson et al., 2005). Both strains were propagated by JABB of the Carolinas (Pine Level, NC) on a solid substrate and formulated in an emulsifiable oil carrier.

Application of Fungal Conidia to Turfgrass

All field studies in 2004 and 2005 were conducted at the BASF field research station in Fuquay-Varina, NC. To obtain baseline ratings of spore viability over time with these previously untested strains, nine 3 x 3 m plots, three for each strain as well as three control plots, were set up on a Bermudagrass research field on 5 Jul 2004 and 8 Aug 2005. The control plot was established to examine the background population of *B. bassiana* conidia present in the soil. Applications of strains 5977 and 3622 at the rate of 5×10^{13} conidia/hectare were made using a CO₂-powered backpack sprayer with an 8003 flat-fan nozzle at 2.8 kg/cm² (Reddick Equipment Co., Williamston, NC). An equivalent amount of water was added to the control plot using the same sprayer (the water was applied to the control plot first to avoid contamination by the conidia treatments). All treatments were arranged in a complete randomized block design with three replicates each. The irrigation system provided approximately 1.3 cm of water to the plots immediately following applications and this same amount of irrigation was applied four times/week throughout the course of the study (Mon, Wed, Fri, Sat). The cumulative amount of water added to this field was approximately 5.1 cm/week. Soil from these plots was classified as sandy loam (humic matter = 1.0%, pH= 6.5, cation exchange capacity = 2.6).

To measure the effect of moisture on spore viability, twelve 3 x 3 m plots were established on two different Bermudagrass fields, directly across a gravel road from one

another (approximately 6 m apart). Six of the plots occurred in the same field as the baseline readings study, while the other six were in the opposite field. The purpose of establishing the plots in this manner was to be able to accurately apply varying amounts of irrigation. The high irrigation regime added approximately 5.1 cm water/week, while the low regime was only irrigated with 2.5 cm water/week. All irrigation occurred on the same four days of the week; the low irrigation regime was obtained by only irrigating for half the amount of time as the high regime field. The soil from the low irrigation field was classified as sandy loam (humic matter = 0.9%, pH= 6.3, cation exchange capacity = 2.7). All treatments were applied on 13 Jul 2004 and 16 Aug 2005 using the same technique and rate as the baseline viability portion of the study, with both strains applied to three replicates in a complete randomized block design in each of the irrigation regime fields. Initial irrigation post application was also applied at the two different levels.

To measure the effect of UV light on spore viability, spores of strain 3622 were formulated in four different carriers that were hypothesized to provide protection from UV degradation. The first treatment was the spores formulated in the proprietary emulsifiable oil carrier from JABB of the Carolinas, Inc.; the other carrier formulations tested were clay, oil + clay (O+C), and oil + optical brightener (OB). Possible advantages for using an oil formulation rather than water only include decreased susceptibility to high temperatures (McClatchie et al., 1994) and decreased sensitivity to UV radiation (Moore et al., 1993; Inglis et al. 1995a). Inglis et al. (1995a) also showed that conidial survival is enhanced by the addition of a water-compatible optical brightener and a clay emulsion. The clay formulation was a wettable powder mixture that consisted of a 10:1 ratio of clay to spores, utilizing a magnesium silicate clay (Microsorb 100 RBM, Englehard Corporation, Iselin,

NJ) that has been shown to effectively overcome the hydrophobic nature of the conidia and get them into suspension in water (Thompson et al., 2005). The oil + clay carrier was formulated as the magnesium silicate clay (10%w/v) in the emulsifiable oil carrier and the oil + optical brightener formulation utilized Tinopal (Calcofluor white M2R, Sigma-Aldrich, St. Louis, MO; 5% w/v) in the same oil carrier. Conidial concentrations were estimated with a hemocytometer and adjusted as necessary to obtain a final concentration 3.375×10^6 conidia/ μ l of formulation. These four sunscreen treatments were arranged as a completely randomized block design in the high irrigation regime field and applied in replicates of three. All applications were made on 21 Jul 2004 and 25 Aug 2005 using the same technique and at the same rate as the baseline and irrigation portions of this study. All weather data for the duration of the three studies in both 2004 and 2005 was compiled by a weather station located onsite at the BASF field research station.

Conidial Suspension Preparation

Preparation of the conidial suspension used for evaluation of viability was the same used by Thompson et al. (2005). This technique involved taking 10-cm cup cutter samples (Lesco, Rocky River, OH) from the plots and using them to prepare the conidial suspension. For all treatments, one sample was taken from each of the three replicated plots on each evaluation date. Each cup cutter sample included the Bermudagrass sod, thatch, and the top 3 cm of soil. The turfgrass samples were combined with 2 liters of water and 2 ml of a citrus emulsifier in a one-gallon plastic jar with a twist-on lid and agitated vigorously for 2 minutes. The liquid-turf solution was then filtered through a series of sieves in the order of No. 12, 100, 170, 325, and 500 (Fisher Scientific, Atlanta, GA). The No. 500 sieve filtered out all soil particles and living

organisms larger than 25µm in size. For all portions of this study, samples were taken at 1, 2, 3, 7, 14, and 21 days after treatment (DAT) for evaluation of conidial viability.

Viability Assessment using Fluorescence Microscopy

Molecular Probes® Live/Dead® BacLight™ Bacterial Viability kit L13152 (Eugene, OR) was used to evaluate the viability of the conidia in the treatment suspensions (Thompson et al. 2005). The kits are composed of a two component staining mixture, the Molecular Probes® SYTO® green-fluorescent nucleic acid stain and the red-fluorescent nucleic acid stain, propidium iodide (Molecular Probes 2001). Concentrations of the two components were used according to a proprietary technique established by JABB of the Carolinas (Anon, 2000). Ten slides were examined for each replication by combining 3.5 µl of the spore suspension and an equal amount of the staining mixture together on a glass slide, stirring with a pipette tip, and covering with a 22 x 22 mm glass cover slip. After 20 minutes in the dark, slides were analyzed at 400x magnification using a Nikon Eclipse E600® Phase Contrast Scope outfitted with a Super High Pressure Mercury Lamp® (Nikon, Tokyo, Japan). Viability was determined according to Schading et al (1995). Conidia that were stained green were considered viable and those that stained red were counted as nonviable. The first 100 conidia encountered on each slide were evaluated and percentage viability was determined from these 100 conidia for each slide. For control slides, effort was made to count all the conidia present by making three horizontal passes across each slide, although 100 conidia were never found on any given slide. Since *B. bassiana* is a naturally occurring soil fungus, all conidia found in the control slides that were similar to the 1.5-4 µ size and the oval shape of *B. bassiana* were included in the determination of the viability of the background population of this fungus in the soil.

Statistical Analysis

For all studies, the mean percentage conidial viabilities for the ten samples in the three replications were analyzed by factorial analysis of variance using the General Linear Models Procedure of SAS (PROC GLM, SAS Institute 2001). For the baseline viability study, comparisons between the mean viabilities of each strain on each date sampled were made using the Tukey's test ($\alpha = 0.05$), with year as a fixed variable and the replications equal to six (three reps over two years). Irrigation treatment effects were determined for each sample date by averaging the conidial viabilities for each strain over both years and using the LSMEANS statement in PROC GLM. For the UV protectant study, comparisons between the mean conidial viabilities of the different carrier formulations on each date sampled were made using the LSD t-test ($P = 0.05$), with year equal to the replication due to a significant year effect for this study.

3. Results

Baseline Study Conidial Viability

Data were analyzed separately for the three studies for all days that samples were taken (1, 2, 3, 7, 14, and 21 DAT) to determine variances in strains or treatments. Background conidia were present in the soil at the BASF research station, although at very low levels. The range of the number of naturally occurring conidia in the control samples was between 0 to 57 conidia found on each slide, with most slides having between 10-30 conidia present. The viability for the background population of conidia present in the control plots ranged from a low of 27.23% to a high of 43.75%. The control values were based on the relatively small number of conidia that were present on each slide, rather than examining the first hundred conidia encountered as with the other samples. No control slide

examined ever contained one hundred conidia that morphologically resembled the size and shape of *B. bassiana* and thus were classified as such. Compared to the hundreds and sometimes thousands of conidia present on the treated slides, the number of naturally-occurring conidia present on the control slides was negligible.

At one DAT, there were significant differences between treatments ($F = 59.89$; $df = 2, 12$; $\alpha = 0.0164$), but this difference was due to the mean conidial viability in control plots being significantly less at 27.23% than either strain 5977 (68.83%) or strain 3622 (68.67%) (Fig.1). At two DAT, significantly fewer conidia were viable in control plots compared to the applied treatments ($F = 19.23$; $df = 2, 12$; $\alpha = 0.0494$), but again there were no differences between strains 5977 and 3622. Both strains experienced approximately 15% mortality between days one and two, with strain 5977 dropping to an average of 50.30% and strain 3622 averaging 53.23% conidial viability (Fig.1). At day three, there were no differences among the strains or the control plot conidia ($F = 8.94$; $df = 2, 12$; $\alpha = 0.1006$), as the mean viabilities of both strains decreased to levels similar to those conidia found in the control plots (Fig.1). There were no significant differences among both strains and the control plots again on day 7 ($F = 0.28$; $df = 2, 12$; $\alpha = 0.7803$), with the mean viability for strain 5977 dropping to 35.25% and strain 3622 to 34.27%. The control stayed in the same range on this evaluation date as all other days in the study with 32.02% of the recovered spores being viable (Fig. 1). This was the same scenario on day 14, with both strains not differing from the control conidial viability ($F = 5.28$; $df = 2, 12$; $\alpha = 0.1592$). However, the viabilities of both strains dropped below the level of the control value (39.27%), ranging from 28.10% (5977) to 35.22% (3622) (Fig. 1). On the last evaluation date (day 21), both strains had dropped to less than 20% viability and were significantly lower the control

sample ($F = 50.60$; $df = 2, 12$; $\alpha = 0.0194$). The two strains did not perform significantly different from one another at any evaluation date and both strains showed very similar declines in viability over time.

Irrigation Study Conidial Viability

Although any naturally occurring population of conidia would be present in all treatment plots as indicated by the control values in the baseline viability study, the small number of spores would not have an impact on the treatment values for the irrigation or UV protectant studies. Differences between irrigation levels were determined within each strain as well as across all strains by averaging over the two strains since there were no differences between the strains in the baseline study at any evaluation date. All four treatments (both strains at the high and low irrigation levels) were very similar at the start of the study, averaging between 70-75% conidial viability (Fig. 2). At one DAT, there was no overall irrigation effect ($F = 2.83$; $df = 1, 16$; $P = 0.1121$), but when analyzing the strains separately, there was an irrigation effect for strain 5977 ($F = 5.73$; $df = 1, 16$; $P = 0.0292$), but not for strain 3622 ($F = 0.00$; $df = 1, 16$; $P = 0.9870$). After two days, there was an overall significant irrigation effect ($F = 44.05$; $df = 1, 16$; $P < 0.0001$), which was also present for both strains, but more pronounced for strain 3622 ($F = 30.36$; $df = 1, 16$; $P < 0.0001$) than strain 5977 ($F = 15.03$; $df = 1, 16$; $P = 0.0013$). The irrigation effect on day three was highly significant overall ($F = 520.03$; $df = 1, 16$; $P < 0.0001$), as well as for both strains (5977: $F = 226.34$; $df = 1, 16$; $P < 0.0001$; 3622: $F = 296.02$; $df = 1, 16$; $P < 0.0001$). This highly significant overall irrigation effect held true throughout the course of the study for all evaluation dates, day 7 ($F = 464.01$; $df = 1, 16$; $P < 0.0001$), day 14 ($F = 618.28$; $df = 1, 16$; $P < 0.0001$) and day 21 ($F = 518.21$; $df = 1, 16$; $P < 0.0001$). The high irrigation regime

maintained conidial viability better than the low irrigation regime, with both strains exhibiting approximately 10% more viability in the high water treatment compared with the low at 3-21 DAT (Fig. 2).

UV Protectants Study Conidial Viability

Only one strain (3622) was used in the UV protectants study, so all treatment differences are due to formulation rather than strain differences. On day one, there were significantly more spores viable in the O+C and OB formulations, which averaged 75.22 and 76.13% viability respectively (Fig. 3), than the oil or clay formulations ($F = 97.41$; $df = 3, 16$; $P < 0.0001$). On this day, the conidia formulated in the oil were also significantly more viable at 70.18% compared to the clay formulation, which had the lowest viability at 64.63%. On day two, the only significant treatment difference was between the O+C and clay carriers ($F = 141.72$; $df = 3, 16$; $P < 0.0001$), with a 10% variation between these two treatments (Fig. 3). On three DAT, the O+C and OB treatments were significantly more viable than the oil and clay carriers ($F = 184.78$; $df = 3, 16$; $P < 0.0001$), which did not differ from one another. By this time, the O+C and OB treatments averaged near 60% viability, while conidia in the oil and clay carriers were only about 48% viable (Fig. 3). This trend continued for the next two evaluation dates and on 7 and 14 DAT the OB and O+C formulations maintained conidial viability at significantly higher levels than the oil or clay carriers ($F = 370.36$; $df = 3, 16$; $P < 0.0001$; $F = 119.21$; $df = 3, 16$; $P < 0.0001$). By 21 DAT, the viabilities for the OB and O+C formulations had decreased to 23.77% and 22.15%, respectively, while only 16.87% of the conidia in the oil carrier and 13.45% of the conidia in the clay carrier were still viable (Fig. 3). The OB and O+C treatments were only

significantly greater in mean percentage conidial viability than the clay carrier and the oil carrier did not differ from any of the other treatments ($F = 191.62$; $df = 3, 16$; $P < 0.0001$).

Weather Data

Both the mean daily air temperatures (Fig. 4) and 5 cm soil temperatures (Fig. 5) remained relatively constant throughout the course of all three studies over both years. Despite the fact that the 2004 studies occurred in Jul and Aug while the 2005 studies took place in Aug and Sept, the overall mean daily temperatures for the duration of the studies were virtually the same (25.43°C in 2004 and 25.59°C in 2005). The lowest mean daily temperatures were 19.3°C in 2004 and 22.6°C in 2005, while the high mean temperatures were 28.4°C and 29.1°C, respectively (Fig. 4). In general, the differences between the low and high mean daily temperatures fell within the relatively small range of 10°C or less (Fig. 4). The mean 5-cm soil temperatures fell into an even smaller range of approximately 5°C (Fig. 5). These temperatures are likely to impact the viability of conidia the most since they more accurately represent the microenvironment of the conidia. The lowest mean soil temperatures were 24.4°C in 2004 and 25.5°C in 2005, while the high mean temperature was 29.8°C for both years (Fig. 5). Like the mean daily air temperature, the overall mean soil temperatures for the duration of the studies were almost identical at 27.63°C in 2004 and 27.23°C in 2005. Although the air and soil temperatures were very similar for the two years, the total rainfall did differ during the course of the studies. There was almost twice the total amount of rain in 2004 compared to 2005; 13.27 cm fell in the first year, while only 6.13 cm of rain occurred in the second year (Fig. 6). The irrigation study would be the most likely study to be impacted by the amount of rainfall. When totaling the rainfall that fell during the course of this test, the difference between years is much less considerable, as 6.64 cm

fell during the irrigation study of 2004 and 4.07 cm during the 2005 irrigation study (Fig. 6), for a difference of only 2.57cm.

4. Discussion

The persistence and maintenance of conidial viability in the field are critical factors influencing the efficacy of entomopathogenic fungi. Although *B. bassiana* strain differences in conidial viability in turfgrass have been documented (Thompson et al. 2005), there were no differences between the baseline viabilities of the two Orthoptera strains evaluated in these tests. The daily mean air and 5-cm soil temperatures were very constant and the total rainfall did not differ dramatically over both years. Regardless of strain, the amount of irrigation applied to field plots treated with conidia enhanced viability over time. Inglis et al. (1995b) showed that simulated rain will wash conidia off of alfalfa and wheat plants and reduce spore persistence by 28-61%. This is probably not the scenario in turfgrass since the application of irrigation will most likely move the conidia into the thatch layer and upper soil profile, the targeted area for root-feeding pests, such as mole crickets. Two different levels of irrigation, supplying totals of 15.3 or 7.5 cm of water, resulted in different conidial viability values on all dates except for 1 DAT. The differences in viability were more significant after three days, and this may be due to the rainfall that fell during the beginning of the study in 2005, which resulted in a minimal difference in actual water applied to the two different irrigation fields during the first few days of the study. The natural levels of rainfall were similar over both years and not significant enough to impact the irrigation effect. If the study had occurred during a year or time period where there was a significant amount of rainfall, the differences between the applied irrigation treatments may have been negated. Although high humidity is not required for spore germination on the insect cuticle

(Ferron, 1977; Ramoska, 1984), freely available water is necessary for conidial survival (Hallsworth and Magan 1999). The relative humidity of a soil atmosphere is likely to be approximately 99% even if the soil is at the permanent wilting point for plants (Griffin, 1963). It is unlikely that the two different irrigation levels resulted in different soil moisture levels, but instead were responsible for the movement of conidia away from the turfgrass surface and exposure to viability-limiting factors such as high temperatures and UV light. We hypothesize that the high irrigation level provided greater protection to the conidia by moving them deeper into the soil profile and away from the abiotic factors that contribute to conidial mortality.

Different carrier formulations will significantly affect conidial viability, with the O+C and OB formulations providing the highest levels of protection for the conidia against UV degradation. Oil formulations have been shown to decrease conidial susceptibility to high temperatures (McClatchie et al., 1994) and UV radiation (Moore et al., 1993; Inglis et al. 1995a). Inglis et al. (1995a) showed that the optical brightener, Tinopal, and an attapulgite clay emulsion would prolong the persistence of *B. bassiana* conidia sprayed on crested wheatgrass. In those tests, the OB and clay treatments were applied as water-soluble additives, and did not contain oil; there was no evaluation of an oil-compatible clay or OB formulation. Our studies supported these findings as the oil formulation performed as well as the clay emulsion, but neither of these carriers supported conidial viability as well as the O+C or OB formulations. Under a phase-contrast microscope, conidia formulated in the O+C carrier appear to be surrounded by clay particles and then enclosed in an oil droplet. Clay acts as a sunlight blocker and provides protection to conidia exposed to UV light (Inglis et al. 1995). That blocking protection along with the absorbing protection of oil

significantly increasing conidial viability. Stilbene brighteners, such as Tinopal, also absorb UV-B radiation (Inglis et al. 1995a) and we attribute the increased conidial viability observed in the OB treatment to the combined protection provided by the oil and the optical brightener.

Finding alternative methods of control for turfgrass insect pests is of great interest given the controversy of using synthetic insecticides to improve plant aesthetics. Unfortunately, biological control agents, as living organisms, are susceptible to many detrimental factors including desiccation and UV damage. These studies support earlier research (Thompson et al. 2005) that *B. bassiana* conidia are capable of surviving for weeks in turfgrass. It appears that higher levels of irrigation will aid the movement of the conidia to locations that provide protection from high temperatures and UV light. Formulating the conidia in carriers that provide UV protection, such as oil-based carriers with a clay or optical brightener additive, will prolong survival in the field. Future research should determine the movement of conidia under various levels of irrigation within the soil profile and also examine the efficacy of different conidial formulations towards mole crickets or other turfgrass pests.

Acknowledgements

The authors gratefully acknowledge Dr. Cavell Brownie in the Department of Statistics, NCSU for her assistance with the statistical analysis of data. Special thanks to David Mann at BASF for all of his help at the field site. The reviews and suggestions of Drs. Wes Watson and Mike Waldvogel are greatly appreciated. Partial support for the completion of this research was provided by the Center for Turfgrass Environmental Research and Education in Raleigh, NC.

References

- Anon. 2000. Standard Operating Procedure (SOP 7-4): Procedure for conidial viability. JABB of the Carolinas, Inc., Pine Level, NC.
- Edgington, S., Segura H., De La Rosa, W., and Williams, T. 2000. Photoprotection of *Beauveria bassiana*: testing simple formulations for control of the coffee berry borer. *Int. J. Pest Manag.* 46(3), 169-176.
- Feng, M.G., Poprawski, T.J. and Khachatourians, G.G. 1994. Production, formulation and application of the entomopathogenic fungus *Beauveria bassiana* for insect control: Current status. *Biocontrol Sci. Technol.* 4, 3-34.
- Ferron, P. 1977. Influence of relative humidity on the development of fungal infection caused by *Beauveria bassiana* (*Fungi Imperfecta*, *Moniliales*) in imagines of *Acanthoscelides obtectus* (Col.: Bruchidae). *Entomophaga* 2, 393-396.
- Firstencel, H., Butt, T.M., and R.I. Carruthers. 1990. A fluorescence microscopy method for determining the viability of entomophthoralean fungal spores. *J. Invert. Pathol.* 55, 258-264.
- Fuxa, J.R. and Richter, A.R. 2004. Effects of soil moisture and composition and fungal isolate on prevalence of *Beauveria bassiana* in laboratory colonies of the red imported fire ant (Hymenoptera: Formicidae). *Env. Entomol.* 33(4), 975-981.
- Goettel, M.S., D.L. Johnson, and Inglis, G.D. 1995. The role of fungi in the control of grasshoppers. *Can. J. Bot.* 73 (Suppl. 1), S71-S75.
- Griffin, D.M. 1963. Soil moisture and the ecology of soil fungi. *Biol. Rev.* 38, 141-166.

- Hallsworth, J.E. and N. Magan. 1999. Water and temperature relations of growth of the entomogenous fungi, *Beauveria bassiana*, *Metarhizium anisopliae*, and *Paecilomyces farinosus*. J. Invert. Pathol. 74, 261-266.
- Hertl, P.T. and R.L. Brandenburg. 1998. Evaluation of *Beauveria bassiana* spore applications for control of mole cricket nymphs in turf. Arthropod Manag. Tests. 23, 323.
- Ignoffo, C.M., Hostetter, D.L., Sikorowski, P.P., Sutter, G., and Brooks, W.M. 1977. Inactivation of representative species of entomopathogenic viruses, a bacterium, fungus, and protozoan by an ultraviolet light source. Env. Entomol. 6, 411-415.
- Inglis, G.D., Goettel, M.S., and Johnson, D.L. 1995a. Influence of ultraviolet light protectants on persistence of the entomopathogenic fungus, *Beauveria bassiana*. Biol. Control 5, 581-590.
- Inglis, G.D., Johnson, D.L., and Goettel, M.S. 1995b. Effects of stimulated rain on the persistence of *Beauveria bassiana* conidia on leaves of alfalfa and wheat. Biocontrol Sci. Technol. 5, 365-369.
- Inglis, G.D., Johnson, D.L., and Goettel, M.S. 1996. Effects of temperature and thermoregulation on mycosis of *Beauveria bassiana* in grasshoppers. Biol. Control 7, 131-139.
- Inglis, G.D., Johnson, D.L., and Goettel, M.S. 1997a. Field and laboratory evaluation of two conidial batches of *Beauveria bassiana* (Balsamo) Vuillemin against grasshoppers. Can. Entomol. 129(1), 171-186.

- Inglis, G.D., Johnson, D.L., and Goettel, M.S. 1997b. Effects of temperature and sunlight on mycosis (*Beauveria bassiana*) (Hyphomycetes: Sympodulosporae) of grasshoppers under field conditions. *Env. Entomol.* 26(2), 400-409.
- Krueger, S. R., Nechols, J.R., and Ramoska, W.A. 1991. Infection of chinch bug, *Blissus leucopterus leucopterus* (Hemiptera: Lygaeidae), adults from *Beauveria bassiana* (Deuteromycotina: Hyphomycetes) conidia in soil under controlled temperature and moisture conditions. *J. Invert. Pathol.* 58, 19-26.
- Lingg, A.J. and Donaldson, M.D. 1981. Biotic and abiotic factors affecting stability of *Beauveria bassiana* conidia in soil. *J. Invertebr. Pathol.* 38, 191-200.
- McClatchie, G.V., Moore, D., Bateman, R.P., and C. Prior. 1994. Effects of temperature on the viability of conidia of *Metarhizium flavoviridae* in oil formulations. *Mycol. Res.* 98, 749-756.
- Moore, D., Bridge, P.D., Higgins, P.M., Bateman, R.P., and Prior, C. 1993. Ultra-violet radiation damage to *Metarhizium flavoviridae* conidia and the protection given by vegetable and mineral oils and chemical sunscreens. *Ann. Applied Biol.* 122, 605-616.
- Morley-Davies, J., Moore, D. and Prior, C. 1995. Screening of *Metarhizium* and *Beauveria* spp. conidia with exposure to simulated light and a range of temperatures. *Mycol. Res.* 100(1), 31-38.
- Molecular Probes® BacLight™ Bacterial Viability Kits Protocol. 2001.
- Ramoska, W.A. 1984. The influence of relative humidity on *Beauveria bassiana* infectivity and replication in the chinch bug, *Blissus leucopterus*. *J. Invert. Pathol.* 43, 389-394.

- SAS Institute. 2003. User's Guide, Version 9.1. SAS Institute, Cary, NC.
- Schading, R.L., Carruthers, R.I., and Mullin-Schading, B.A. 1995. Rapid determination of conidial viability for entomopathogenic hyphomycetes using fluorescence microscopy techniques. *Biocontrol Sci. Technol.* 5, 201-208.
- Studdert, J.P. and Kaya, H.K. 1990. Water potential, temperature, and clay-coating of *Beauveria bassiana* conidia: effect on *Spodoptera exigua* pupal mortality in two soil types. *J. Invert. Pathol.* 56, 327-336.
- Thompson, S.R., Brandenburg, R.L., and Arends, J.J. 2005. Conidial viability and pathogenicity of *Beauveria bassiana* (Balsamo) Vuillemin for mole cricket (Orthoptera: Gryllotalpidae) control in turfgrass. *Int. Turfgrass Soc. Res. J.* 10, 784-792.
- Xia, Y., P.T. Hertl, and R.L. Brandenburg. 2000. Surface and subsurface application of *Beauveria bassiana* for controlling mole crickets (Orthoptera: Gryllotalpidae) in golf courses. *J. Agric. Urban Entomol.* 17, 177-189.

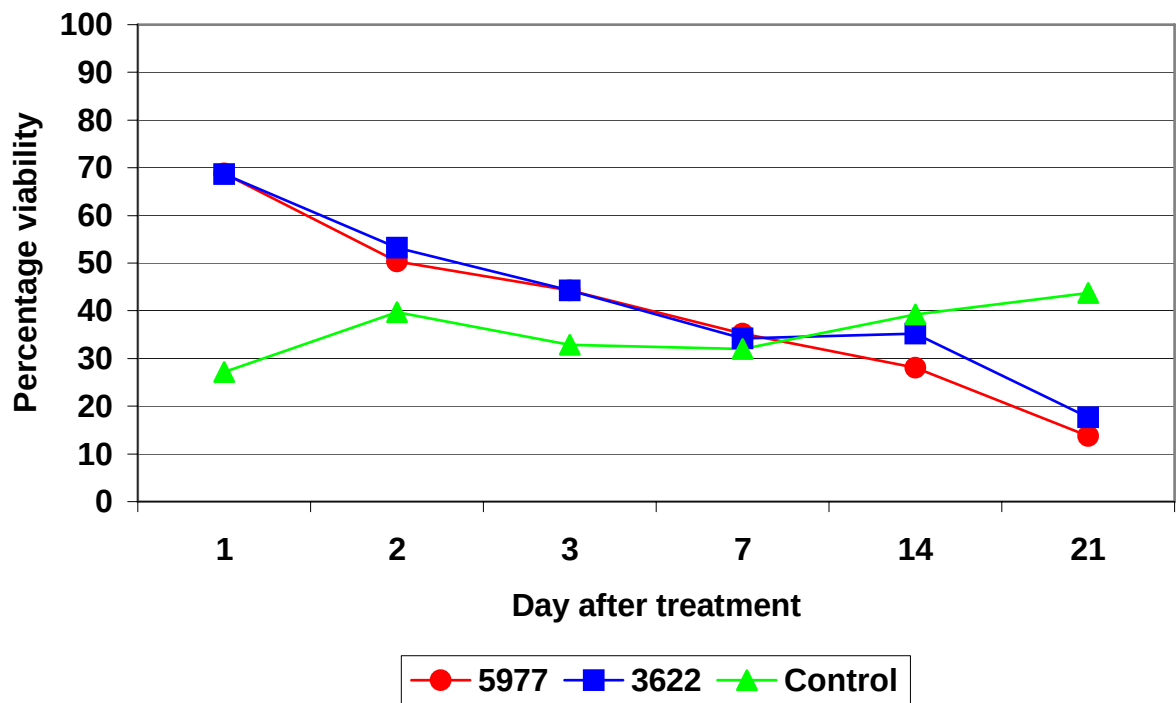


Fig. 1. Mean percentage conidial mortality for *B. bassiana* strains 5977 and 3622 over time (BASF field research station, Fuquay-Varina, NC).

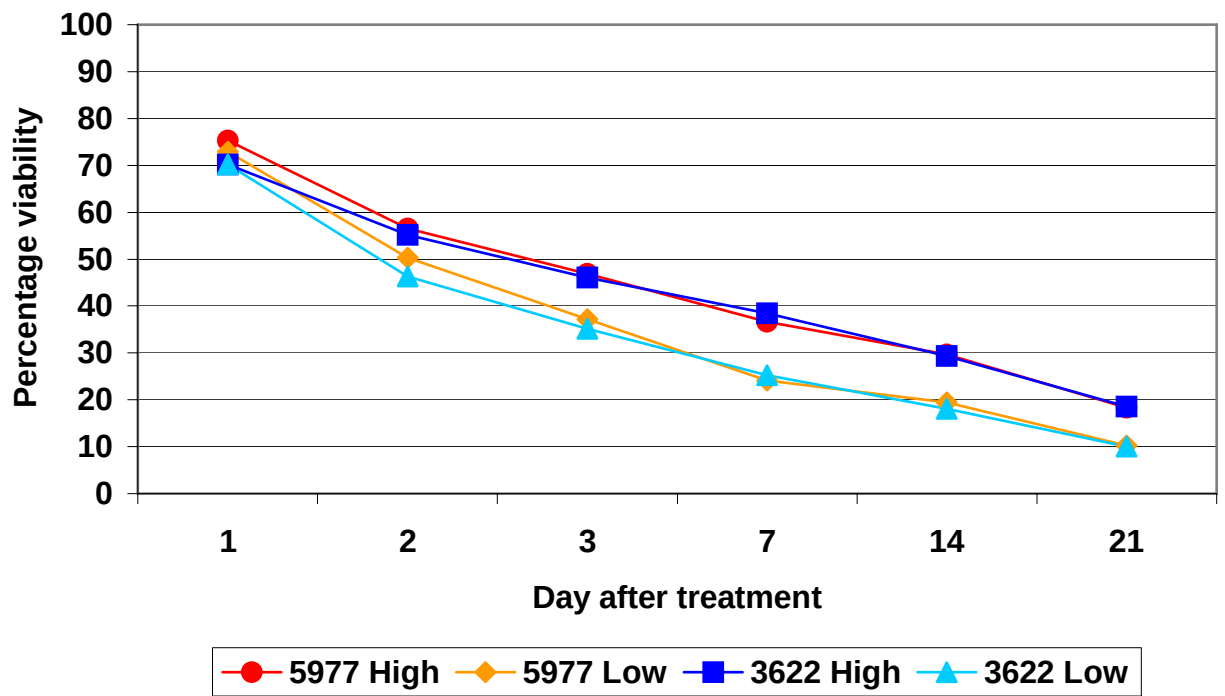


Fig. 2. Mean percentage conidial viability for *B. bassiana* strains 5977 and 3622 at two different irrigation regimes (high and low) over time (BASF field research station, Fuquay-Varina, NC).

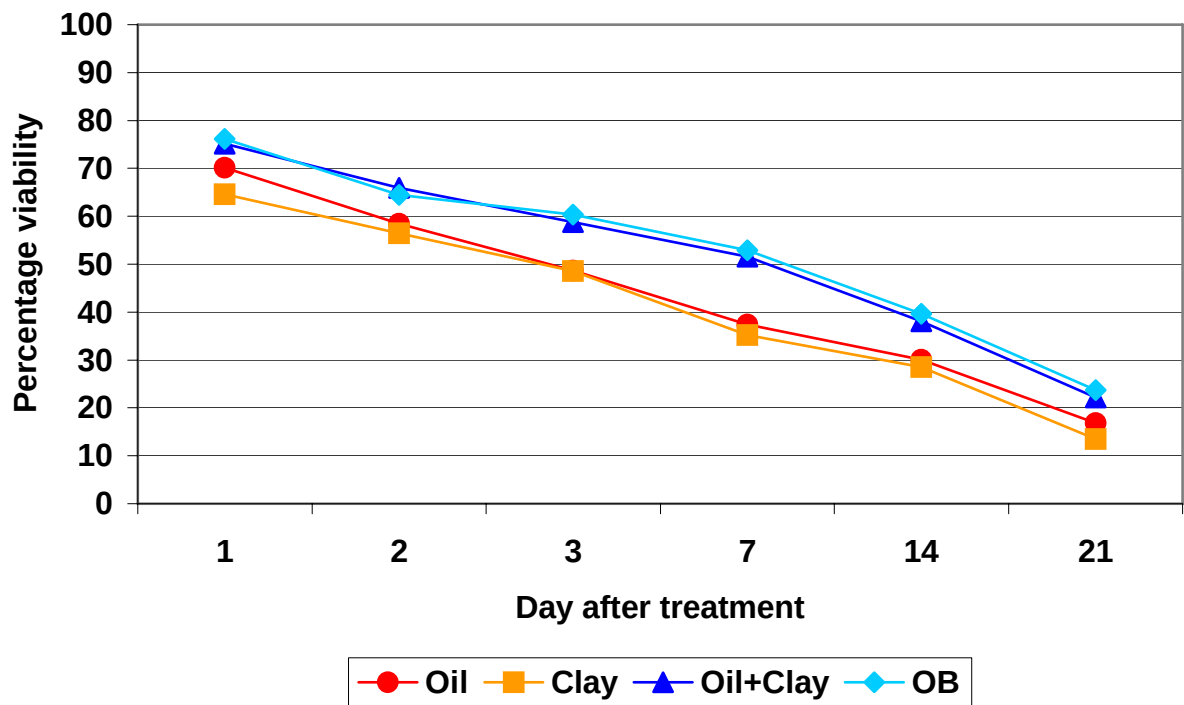


Fig. 3. Mean percentage conidial viability for four different UV protectant formulations of *B. bassiana* strain 3622 over time (BASF field research station, Fuquay-Varina, NC).

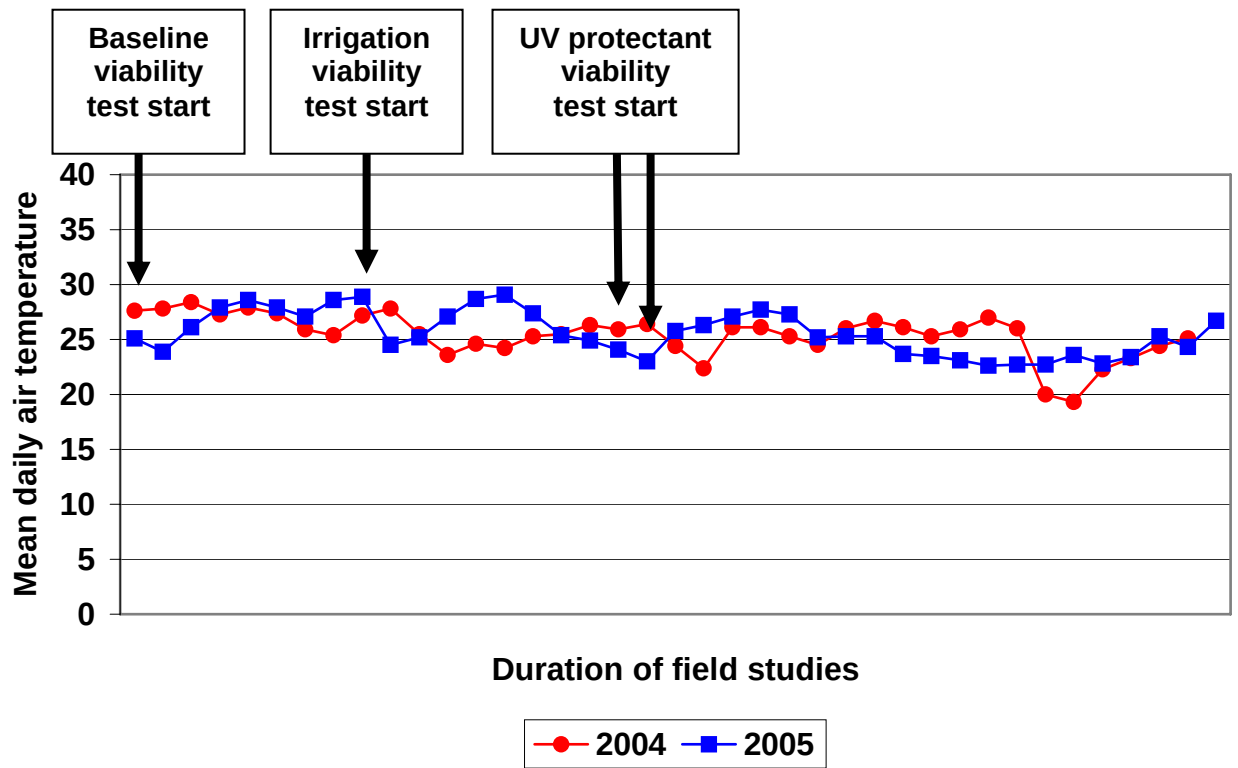


Fig. 4. Mean daily air temperature (°C) during 2004 and 2005 field studies, indicating start of baseline, irrigation, and UV protectant viability tests. 2004 data include temperatures from 5 Jul to 11 Aug; 2005 data include temperatures from 8 Aug to 15 Sep.

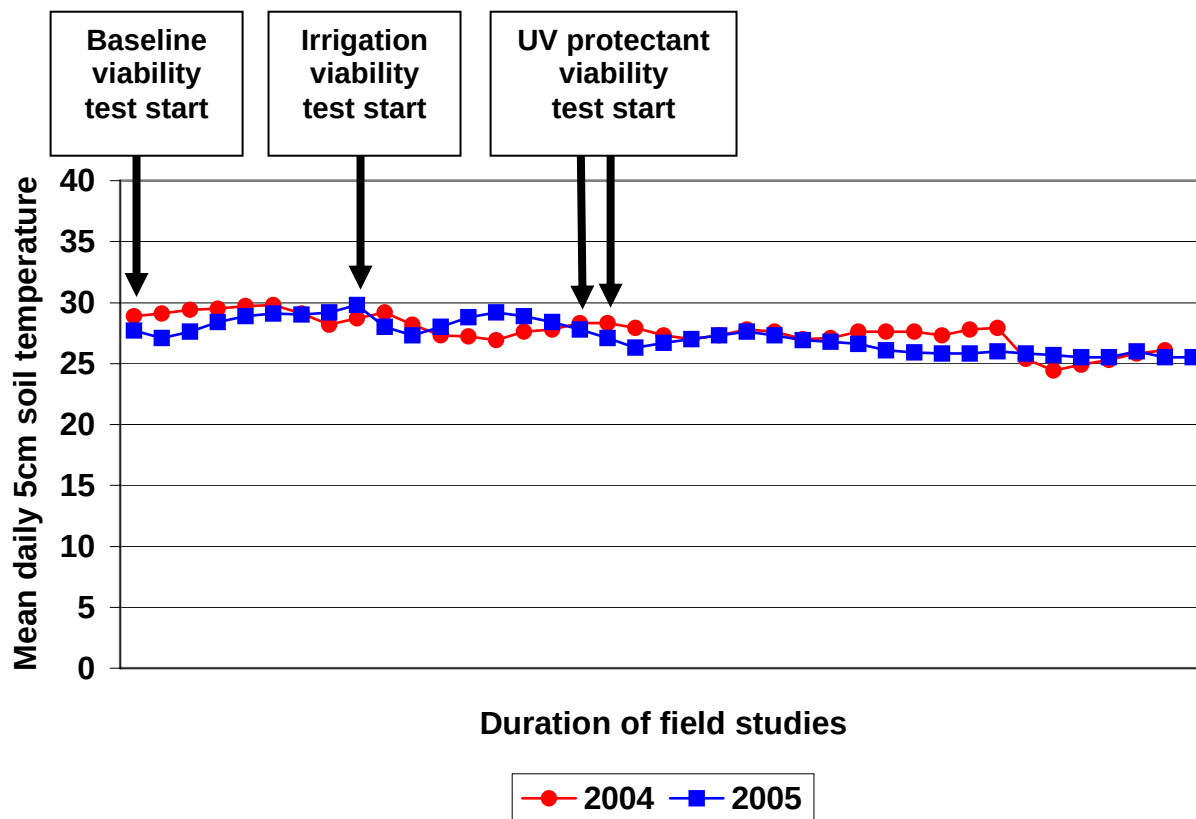


Fig. 5. Mean daily soil temperature (°C) at depth of 5 cm during 2004 and 2005 field studies, indicating start of baseline, irrigation, and UV protectant viability tests. 2004 data include temperatures from 5 Jul to 11 Aug; 2005 data include temperatures from 8 Aug to 15 Sep.

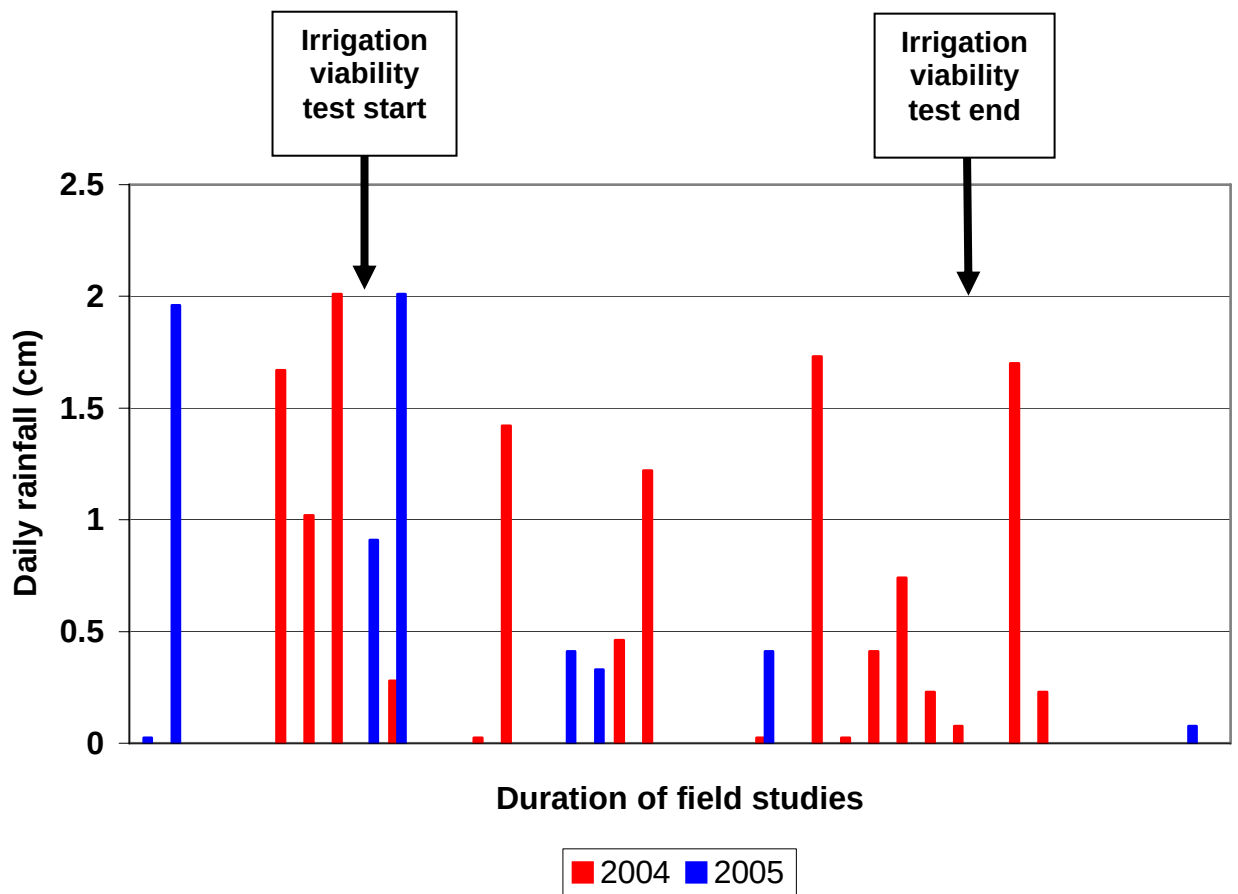


Fig. 6. Daily rainfall amounts (cm) during 2004 and 2005 field studies, indicating start and end of irrigation viability test. 2004 data include temperatures from 5 Jul to 11 Aug; 2005 data include temperatures from 8 Aug to 15 Sep.

J. Economic Entomology

Sarah Rachel Thompson

Biological and Microbial Control

North Carolina State University

Thompson and Brandenburg: *B. bassiana*- Imidacloprid/

Department of Entomology

Diatomaceous Earth Interactions

Box 7613

Raleigh, NC 27695-7613

Phone: (919) 513-2209

Fax: (919) 515-7746

sarah_thompson@ncsu.edu

**EFFECT OF COMBINING IMIDACLOPRID AND DIATOMACEOUS EARTH
WITH *BEAUVERIA BASSIANA* (BALSAMO) VUILLEMIN ON MOLE CRICKET
(ORTHOPTERA: GRYLLOTALPIDAE) MORTALITY**

SARAH R. THOMPSON AND RICK L. BRANDENBURG

Department of Entomology, North Carolina State University, Box 7613, Raleigh, NC 27695

Abstract

Sublethal doses of three different Orthoptera-derived strains of *Beauveria bassiana* (Balsamo) Vuillemin were topically applied to adult southern mole crickets, *Scapteriscus borellii* Giglio-Tos, and tested in combination with substrate treatments of diatomaceous earth (DE) and imidacloprid. Crickets treated only with the high rates (10^8 conidia/cricket) of each of the three *B. bassiana* strains exhibited the shortest survival times as well as the highest percentage mortality at 28 days after treatment. However, these treatments did not differ significantly from any of the diatomaceous earth combination treatments. Two of the strains tested, 5977 and 3622, exhibited synergistic interactions with DE. While the third strain, GHA, was not significant for synergy, the mortality caused by the combination treatment was still greater than the expected additive effect. DE abrades the insect cuticle and absorbs cuticular lipids, aiding the entry of germinating conidia into the mole cricket hemocoel. None of the three strains exhibited synergy when combined with imidacloprid, and mortality of all combination treatments was less than additive. For strain 5977, there was an antagonistic interaction with imidacloprid. It was difficult to obtain less than 30% mortality for imidacloprid only treatments, which was considered the upper limit for sublethal doses. The mean percentage mortality caused by imidacloprid was 37.5%, and this high percentage made it difficult for any combination treatment to cause significantly more mortality than the expected additive effect. These results clarify the interactions of other control products with *B. bassiana* and provide a basis for a reduced pesticide approach to mole cricket control.

KEY WORDS: *Beauveria bassiana*, diatomaceous earth, imidacloprid, *Scapteriscus borellii*, synergy

Introduction

Mole crickets (Orthoptera: Gryllotalpidae) are considered to be one of the most important groups of turf and pasture grass pests in the southeastern U.S. (Walker and Ngo 1982). Damage is caused by the feeding on the grass roots as well as the production of underground and surface tunnels, which lead to desiccation of the root zone and mechanical damage to the turfgrass (Villani et al. 2002). The subterranean nature of mole crickets requires high rates and repeated applications of synthetic insecticides to achieve acceptable levels of control (Xia et al. 2000). A large proportion of highly managed turfgrass is in close proximity to environmentally-sensitive areas such as irrigation lakes, wildlife habitats, and residences (Thompson et al. 2005). Public concern over the possible negative effects of chemical insecticides has led to a demand for research on more environmentally-safe control options, such as microbial agents (Goettel et al. 1995). The move to find alternative control agents is especially necessary in turfgrass and ornamental research, a field where many individuals, organizations, and government agencies have questioned the use of synthetic pesticides for aesthetic enhancement.

A potential alternative to synthetic insecticides is the use of biological control agents, such as pathogenic fungi. *Beauveria bassiana* (Balsamo) Vuillemin is one of the most commonly studied entomopathogenic fungi with pathogenicity to over 200 different species of insects (Feng et al. 1994). Fungi-infected mole crickets have been observed by many different researchers (Boucias 1985, Hazarika et al. 1994, Hudson et al. 1988, Thompson unpublished data), but otherwise, relatively little research has been conducted using this pathogen for mole cricket control. A field study evaluating surface or subsurface application of *B. bassiana* for mole cricket control suggests that repeated applications are needed to

minimize or eliminate signs of damage (Xia et al. 2000). Another field study failed to show significantly less mole cricket damage in plots treated with *B. bassiana* alone or in combination with imidacloprid compared to untreated plots (Hertl and Brandenburg 1998). However, laboratory studies have confirmed pathogenicity of selected strains of *B. bassiana* towards mole crickets, with those strains isolated from close relatives causing the most mortality in the shortest time (Thompson et al. 2005). This research supports the hypothesis that strains of pathogenic fungi isolated from the original host or a closely related species are the most virulent (Xu 1988) and that genotypes of *B. bassiana* exhibit varying levels of pathogenicity towards different insect hosts (Jaronski and Goettel 1997). In addition to adequate host targeting, the performance of *B. bassiana* is dependent on the ability of the fungus to persist in the microenvironment (Inglis et al. 1997). One important factor that determines the persistence and maintenance of conidial viability is high humidity, which supports fungal germination.

Significant production costs and high application rates have made the control of mole crickets with *B. bassiana* economically impractical on a large-scale basis. An alternative option for pest control that is efficacious under low humidity levels, is the use of desiccant dusts, such as diatomaceous earth (DE). DE appears to cause abrasion and lipid adsorption on the insect cuticle (Ebeling 1971). Cuticular abrasion may enhance the efficacy of *B. bassiana* since the mode of entry into the insect's hemocoel is cuticular penetration. Removal of cuticular phospholipids and long-chain fatty acids by DE increases conidial attachment and makes more nutrients available for the conidia (Akbar et al. 2004). The complimentary moisture optima and ability of DE to aid the penetration of hyphae through the insect cuticle suggest the potential for synergism between the two products

(Akbar et al. 2004, Lord 2001). Research has shown a synergistic interaction between DE and *B. bassiana* for control of larval red flour beetles, *Tribolium castaneum* (Akbar et al. 2004), and that DE has no negative effect on germinating conidia (Lord 2001). One of the objectives of our research was to test for synergy between DE and *B. bassiana* on mole crickets, in an attempt to identify another alternative control product that could be used in conjunction with the fungus to lower application rates and increase the feasibility of large-scale usage.

The second objective was to test whether *B. bassiana* acted additively, synergistically, or antagonistically with another commonly used conventional turfgrass insecticide, imidacloprid. Imidacloprid is often applied in managed turfgrass to control white grub complexes, and therefore, would possibly be present when *B. bassiana* spore applications were made for nymphal mole cricket control. Varying results have been obtained when combining this chloronicotinyl insecticide and fungus; some reports have shown synergistic effects on mortality (Furlong and Groden 2001) and mycosis (Boucias et al. 1996, Quintela and McCoy 1997a) of different insect species, while other research has shown less than additive or antagonistic interactions when the two products are used in combination (James and Elzen 2001). Although most of the studies exposed the insects to imidacloprid through an oral route due to its systemic nature, Quintela and McCoy (1997a) observed a synergistic response with a contact treatment as well. Muscular paralysis led to a loss of larval mobility for *Diaprepes abbreviatus*, which interfered with conidial avoidance behaviors in the substrate (Quintela and McCoy 1997b) and was attributed to the increased response. Since mole cricket avoidance behaviors towards *B. bassiana* have been observed (Thompson and Brandenburg 2005), we wanted to clarify this interaction between *B.*

bassiana and imidacloprid on the previously untested southern mole cricket, *Scapteriscus borellii* Giglio-Tos.

Materials and Methods

Propagation of Fungal Isolates. Two of the strains of *B. bassiana* used in this study, 5977 and 3622 were obtained as cultures from the ARS Collection of Entomopathogenic Fungal Cultures (Ithaca, NY). Strain 5877 is IMI (331275) isolate and its origination is specified as Orthoptera with no specific location. J.L. Stimac isolated strain 3622 in Florida from the tawny mole cricket, *Scapteriscus vicinus*. These strains were chosen since both originate from orthopteran hosts and previous research indicates that strains isolated from a close relative of the target host are the most virulent (Thompson et al. 2005). Both strains were propagated by JABB of the Carolinas (Pine Level, NC) on a solid substrate. The third strain of *B. bassiana*, strain GHA, was obtained from Emerald Bioagriculture Corporation (Butte, MT). The two treatments used in conjunction with *B. bassiana* were the commercially available turfgrass insecticide, imidacloprid (Merit® 75 WSP, Bayer Corporation, Research Triangle Park, NC) and DE crawling insect killer (Concern®, Woodstream Corporation, Lititz, PA).

Collection of Mole Crickets. Adult *S. borellii* were collected from four golf courses (The Lakes Country Club in Boiling Springs Lakes, Sandpiper Bay Golf Course in Sunset Beach, Olde Fort Golf Club in Wilmington, and Duck Golf Club also in Wilmington) in New Hanover and Brunswick Counties, NC during the months of March through May in 2004 and 2005. Crickets were attracted to electronic callers (Ulagaraj and Walker 1973) that mimic the male mole cricket calls, which are produced in the evenings during the spring months to attract females for mating (Ulagaraj 1976). The callers were suspended above a

modified pool catch trap, which utilized two plastic wading pools, one placed on top of the other (Thompson and Brandenburg 2004). The pool on top had holes cut into it, through which the crickets fell once they flew to the callers, and the bottom was filled with moist sand to hold the crickets until they were harvested. Crickets were collected on a weekly basis during these three months for both years. All crickets were held individually to prevent cannibalism and maintained in soil from The Lakes Country Club collection site (classified as Wando fine sand, HM = 0.36%, pH = 6.2, CEC = 4.5) at 10% moisture in a climate-controlled laboratory on the campus of North Carolina State University (Raleigh, NC). The crickets were fed fishing-bait earthworms (Variety Pickup, Raleigh, NC) until the topical bioassays were performed.

Pre-treatment of Substrate. Two days prior to treatment of the mole crickets with conidia, the substrate was pre-treated with either DE or imidacloprid. Previous topical bioassays identified rates of both products that produced less than 30% mortality and these rates were classified as sublethal doses. For imidacloprid, the sublethal dose for mole crickets was determined to be 3 ppm and for DE the sublethal dose was 75g of product/kg substrate. Plastic containers (473 ml, Reynolds, Richmond, VA) were filled with 10% moisture (wt/wt) Metro Mix® (The Scotts Company, Marysville, OH), a combination of perlite, vermiculite, peat moss, and sand. Since conidial attachment is necessary for mycosis (Brandenburg and Villani 1995), Metro Mix® was used rather than more abrasive field-collected soil. It was thought that this substrate would be less likely to remove conidia from the mole cricket external cuticle as they tunneled. The Metro Mix® was autoclaved and all containers were sterilized prior to use in a 10% bleach solution (6% sodium hypochlorite, The Clorox Company, Oakland, CA) to eliminate cross contamination by other fungi or

bacteria. For the controls, *B. bassiana* alone treatments, and all DE treatments, the 10% moisture level of the substrate was obtained by adding sterile, distilled water to the Metro Mix®. For all treatments involving imidacloprid, the moisture level was obtained from the wettable powder formulation. One adult mole cricket was then added to each substrate-filled container, whether pre-treated or not, as was the situation for those treatments that did not involve either imidacloprid or DE (controls and all *B. bassiana* alone treatments for each strain). In 2004, the substrate treatments were conducted on 31 May and in 2005, these treatments were performed on 6 Jun.

Treatment of Mole Crickets with Fungus. Two days after substrate treatment, the mole crickets were removed from their respective containers and treated with known numbers of conidia of the three strains of *B. bassiana* that were mixed with 0.13 mg magnesium silicate clay (Microsorb 100 RBM, Englehard Corporation, Iselin, NJ) and 10 ml of sterile distilled water. The addition of clay prevented clumping of the conidia without directly causing mortality of the crickets. The conidia, clay, and water mixture was agitated to formulate suspensions consisting 5×10^7 or 5×10^8 conidia. The crickets were treated in groups of 5 at a time. Thompson et al. (2005) had previously determined that 10^7 conidia/cricket was a sublethal dose and 10^8 conidia/cricket was a lethal dose for most *B. bassiana* strains. Two sets of controls were performed, one with 200 ml of sterile distilled water, and the other with 190 ml of sterile distilled water plus 10 ml of a sterile distilled water and 0.13 mg clay suspension. The crickets from the imidacloprid and DE treatments that did not include *B. bassiana* were also treated with 200 ml of sterile distilled water. The technique used to treat the crickets with the conidia was identical to that of a previous study (Thompson et al. 2005). This technique involved pouring the 10 ml conidial suspension

added to 190 ml of sterile distilled water on top of the crickets that were placed in a 11.0 cm Büchner funnel. The funnel was lined with filtered paper and suspended over 1000 ml Erlenmeyer flask, which allowed for the liquid to be suctioned off after 30 seconds (Castrillo and Brooks 1998). The water and water plus clay controls were performed first to eliminate potential spore contamination in the funnel. Each treatment was replicated four times for a total number of 20 crickets per treatment. The crickets were returned to their respective treated-substrate containers after exposure to the conidial suspension. In total, the treatments evaluated in this bioassay included: water and clay controls, pathogenic rates of each of the three *B. bassiana* strains (10^8 conidia/cricket), sublethal rates of each strain (10^7 conidia/cricket), sublethal doses of imidacloprid and DE, combination treatments of imidacloprid with each of the three strains, and combination treatments of DE with each strain. All combination treatments were made using sublethal doses of each product to test for synergy. Earthworms were added as food to containers as necessary and 15 ml of water was added every 7 days throughout the observation period to maintain substrate moisture levels. Crickets were checked daily for mortality for 28 days.

Incubation of Cadavers. Dead mole crickets were removed from the Metro Mix® filled containers with sterile forceps, surface-sterilized, and transferred to 118 ml Nalgene® containers with plastic screw-on lids. Surface sterilization and incubation was performed according to Thompson et al. (2005). All Nalgene® containers and screens were sterilized in 10% bleach (6% sodium hypochlorite, The Clorox Company, Oakland, CA) solution before use to prevent contamination. Containers were sealed and placed in an incubator at 27 C° to allow for sporulation of the fungus (Hallsworth and Magan 1999). All cadavers were checked daily for signs of conidial bloom, which appears as a muscardine white fungus

covering the insect body (Jaronski and Goettel 1997). The substrate pre-treatment, exposure to fungus, observation for mortality, and incubation of cadavers was conducted in the same manner in 2004 and 2005.

Statistical Analysis. Data from these studies were analyzed as mean percentage mortality at 28 days after treatment (DAT) as well as mean survival time, which is a conservative estimate of the length of time for the crickets to die accounting for both censored (mole crickets that were still living at day 28) and non-censored values (the day that dead mole crickets died). Data were analyzed by factorial analysis of variance using the General Linear Models Procedure of SAS (SAS Institute, Cary, NC), which averages the data over the two years and uses the eight batches as the reps (4 batches each year). Comparisons between the mean 28-day mortalities and mean survival times were made using the LSD t-test ($P = 0.05$). Synergy was defined as a significant departure from zero in this equation: $((B. \textit{bassiana} + \text{DE/Imid}) - B. \textit{bassiana}) - (\text{DE/Imid} - \text{Clay}) = 0$ where DE/Imid represents the effect of sublethal DE (or imidacloprid) treatment; Clay represents the effect of the clay control treatment; $(B. \textit{bassiana} + \text{DE/Imid})$ represents the effect of the combination treatment (conducted for each strain plus either DE or imidacloprid); and *B. bassiana* represents the effect of the sublethal *B. bassiana* treatment (for each strain, respectively). Tests for synergy were made using contrast statements in PROC GLM (SAS 2003).

Results

There was a significant difference in survival times among the treatments ($F = 15.31$; $df = 15, 512$; $P < 0.0001$), with the mean survival times for both control treatments, water and clay, averaging over 25 days. The survival times of both DE and imidacloprid at

sublethal doses did not differ from either control treatment and averaged at least 20 days (Table 1). The sublethal dose of *B. bassiana* (10^7 conidia/cricket), regardless of the strain, also did not differ from the control treatments in respect to mean survival time, averaging between 18 and 23 days (Table 1). All three *B. bassiana* treatments at the high rate of 10^8 conidia/cricket exhibited survival times significantly shorter than the controls, as did all three combination treatments involving *B. bassiana* strains and DE (Table 1). The results were mixed with imidacloprid combination treatments, with only those treatments involving strains 3622 and GHA exhibiting shorter survival times compared to the controls (Table 1). The overall shortest survival times were observed in the high rate treatments of *B. bassiana*, the low rate of *B. bassiana* strain GHA, all DE combination treatments, and the imidacloprid combination treatments involving strains 3622 and GHA (Table 1). Overall, the survival times from the 2004 test were shorter than those observed in 2005 for the same treatments, but the differences were especially pronounced in all 10^8 conidia/cricket *B. bassiana* treatments.

There were significant differences among treatments for mean percentage mortality at 14 DAT ($F = 7.41$; $df = 15, 112$; $P < 0.0001$) as well as 28 DAT ($F = 18.37$; $df = 15, 96$; $P < 0.0001$). Comparisons among treatments will be discussed for the 28 DAT analysis (Figs. 1-3). The water control resulted in an average of 10% mortality in each batch, which did not differ significantly from the clay control mean percentage mortality, 15%. For strain 5977, the high rate and the combination treatment with DE caused the most mortality, 92.5% and 82.5%, respectively, and were significantly greater than all other treatments (Fig. 1). The strain 5977 combination treatment with DE caused significantly more mortality than either treatment alone; the sublethal dose of this strain caused 32.5% and DE caused 27.5%

mortality at 28 DAT (Fig. 1). This combination treatment was significant in the test for synergy (Table 2). The combination treatment with imidacloprid did not cause significantly more mortality (52.5%) than the imidacloprid alone treatment (37.5%) (Fig. 1), and was not significant in the test for synergy (Table 2). In fact, because the estimate and t value were both negative for this analysis (Table 2), it suggests an antagonistic relationship between the two products, since the mean percentage mortality was less than the expected additive effect.

For strain 3622, the combination treatment with DE caused the most mortality at 28 DAT (87.5%), which was significantly greater than all other treatments except for the high rate of the fungus alone (85.0%) (Fig. 2). The high rate of strain 3622 did not differ from the combination treatment with imidacloprid, which caused 67.5% mortality, but the two combination treatments were still significantly different from one another (Fig. 2). There was a synergistic relationship between this strain and DE, but not with imidacloprid (Table 2). For this strain, there is no evidence of an antagonistic relationship with imidacloprid.

GHA, which is commercially available from Emerald Bioagriculture (Butte, MT), showed similar results as to what was observed with the other two strains. There was no significant difference between the mean percentage mortality that was caused by the high dose of this strain (82.5%), the combination treatment with DE (82.5%), and the combination treatment with imidacloprid (70.0%) (Fig. 3). However, the combination treatments caused significantly more mortality than either component, DE (or imidacloprid), or the low rate of the fungus (Fig. 3). There was an indication of synergism between this strain and DE, but the test was only borderline significant (Table 2). On the other hand, the combination of GHA and imidacloprid did not exhibit synergism or antagonism (Table 2).

Discussion

The high rates of all three *B. bassiana* strains caused more overall mortality than any of the combination treatments involving imidacloprid. This may be a result of the mole crickets not feeding on the systemic insecticide as was the case in most other tests involving the fungus and imidacloprid (Boucias et al. 1996, Furlong and Groden 2001, James and Elzen 2001); however, Quintela and McCoy (1997a) observed a synergistic interaction when *B. bassiana* was combined with sublethal doses of imidacloprid as a contact or oral treatment. The antagonism reported by James and Elzen (2001) was attributed to a behavioral response caused by *B. bassiana* that inhibited feeding and uptake of imidacloprid. Since the route of exposure in our study was through substrate treatment, this hypothesis cannot explain the less than additive effect seen with strains 3622 and GHA and antagonistic interaction with strain 5977. One possible explanation for these results is the difficulty in obtaining an accurate sublethal dose of imidacloprid for adult mole crickets. Various topical bioassays were performed prior to undertaking these tests to identify a rate of imidacloprid that causes < 30% mortality in mole crickets when applied as a substrate treatment. After two different bioassays revealed that 3 ppm achieved this desired result, this rate was chosen as the sublethal dose. But, the mean percentage mortality caused by this rate in the 2004 and 2005 tests was actually 37.5%, making it difficult for any of the combination treatments to result in mortality rates significantly greater than the expected additive mortality rates of 70.0% (with strain 5977), 62.5% (with strain 3622), or 82.5% (with strain GHA). Additionally, although avoidance behaviors have been documented when mole crickets are exposed to *B. bassiana* conidia (Thompson and Brandenburg 2005), no mole cricket grooming behaviors have been observed which could suggest an intentional

removal of conidia from the cuticle, as is the case with termites (Boucias et al. 1996). The muscular paralysis that occurs after exposure to imidacloprid has been suggested to inhibit the removal of conidia from the cuticle through these grooming behaviors and thus enhance virulence of the fungus. As a result, there is no clear reason for why a combination treatment of sublethal doses of imidacloprid and *B. bassiana* would result in pathogenicity enhancement to mole crickets and our study confirms that a synergistic interaction does not occur.

In contrast, the abrasive nature of DE would appear to aid with the mechanical entry of the germinating conidia into the mole cricket hemocoel. Also, since DE absorbs lipids on the insect cuticle (Lord 2001), it may make nutrients more available to germinating conidia (Akbar et al. 2004) and enhance the virulence of the fungus. Two of the three *B. bassiana* strains (5977 and 3622) tested exhibited a synergistic relationship when combined with diatomaceous earth, and the test for synergism involving the third strain (GHA) approached significance as well. These results support the work of Lord (2001) and Akbar et al. (2004), who also observed synergism when combining *B. bassiana* treatments with DE for control of stored-grain beetles. None of the DE combination treatments were significantly different from the high rate *B. bassiana* treatments in regards to survival time or percentage mortality at 28 DAT, indicating that the enhanced pathogenicity applies to both overall mortality and time till death, both of which are critical properties of any insecticide. Enhancing the pathogenicity of *B. bassiana* would make the use of this product more economically feasible on a commercial scale since the combination treatment of sublethal doses of each product produces the same effect as the high rate of the fungus. The contrasting moisture optima of these two products may also allow for insect mortality at all moisture levels. Since both *B.*

bassiana and DE are environmentally-safe control products, the results from this work suggest a reduced insecticide approach for mole cricket control and provide insight into ways to enhance the efficacy of entomopathogenic fungi.

Acknowledgements

The authors gratefully acknowledge Dr. Cavell Brownie in the Department of Statistics, NCSU for her assistance with the statistical analysis of data. Special thanks to the superintendents/owners of all four golf courses for allowing the (loud) calling traps to function at those locations during the mole cricket collection periods. The reviews and suggestions of Drs. Watson and Kennedy are greatly appreciated. Partial support for the completion of this research was provided by the Center for Turfgrass Environmental Research and Education in Raleigh, NC.

References Cited

- Akbar, W., J.C. Lord, J.R. Nechols, and R.W. Howard. 2004. Diatomaceous earth increases the efficacy of *Beauveria bassiana* against *Tribolium castaneum* larvae and increases conidia attachment. J. Econ. Entomol. 97(2): 273-280.
- Boucias, D.G. 1985. Diseases. pp. 32-35. In T.J. Walker (ed.) Mole crickets in Florida. Fla. AES. Bull. 846.
- Boucias, D.G., C. Stokes, G. Storey, and J.C. Pendland. 1996. The effects of imidacloprid on the termite *Reticulitermes flavipes* and its interaction with the mycopathogen *Beauveria bassiana*. Pflanzenschutz-Nachr. Bayer. 49: 103-144.
- Brandenburg, R.L. and M.G. Villani. 1995. Handbook of Turfgrass Pests. Entomological Society of America, Lanham, MD.
- Castrillo, L.A. and W.M. Brooks. 1998. Differentiation of *Beauveria bassiana* isolates from the darkling beetle, *Alphitobius diaperinus*, using isozyme and RAPD analyses. J. Invert. Path. 72: 190-196.
- Ebeling, E. 1971. Sorptive dusts for pest control. Annu. Rev. Entomol. 16: 123-158.
- Feng, M.G., T.J. Poprawski, and G.G. Khachatourians. 1994. Production, formulation, and application of the entomopathogenic fungus *Beauveria bassiana* for insect control: current status. Biocon. Sci. Tech. 4: 3-34.
- Furlong, M.J. and E. Groden. 2001. Evaluation of synergistic interactions between Colorado potato beetle (Coleoptera: Chrysomelidae) pathogen *Beauveria bassiana* and the insecticides, imidacloprid, and cyromazine. J. Econ. Entomol. 94(2): 344-356.

- Goettel, M.S., D.L. Johnson, and G.D. Inglis. 1995. The role of fungi in the control of grasshoppers. *Can. J. Bot.* 73 (Suppl. 1): S71-S75.
- Hallsworth, J.E. and N. Magan. 1999. Water and temperature relations of growth of the entomogenous fungi, *Beauveria bassiana*, *Metarhizium anisopliae*, and *Paecilomyces farinosus*. *J. Invert. Path.* 74: 261-266.
- Hazarika, L.K., K.C. Puzari, and G. Baruah. 1994. Fungi as microbial insecticides on mole cricket. *Two and a Bud.* 41(2): 22-25.
- Hertl, P.T. and R. L. Brandenburg. 1998. Evaluation of *Beauveria bassiana* spore applications for control of mole cricket nymphs in turf, 1997. *Arthropod Man. Tests.* 23: 323.
- Hudson, W.G., J.H. Frank, and J.L. Castner. 1988. Biological control of *Scapteriscus* spp. mole crickets (Orthoptera: Gryllotalpidae) in Florida. *Bull. Entomol. Soc. Am.* 34(4): 192-198.
- Inglis, G.D., D.L. Johnson, and M.S. Goettel. 1997. Field and laboratory evaluation of two conidial batches of *Beauveria bassiana* (Balsamo) Vuillemin against grasshoppers. *Can. Entomol.* 129(1): 171-186.
- James, R.R. and G.W. Elzen. 2001. Antagonism between *Beauveria bassiana* and imidacloprid when combined for *Bemisia argentifolii* (Homoptera: Aleyrodidae) control. *J. Econ. Entomol.* 94(2): 357-361.
- Jaronski, S.T. and M.S. Goettel. 1997. Development of *Beauveria bassiana* for control of grasshoppers and locusts. *Mem. Entomol. Soc. Can.* 171: 225-237.

- Lord, J.C. 2001. Desiccant dusts synergize the effect of *Beauveria bassiana* (Hyphomycetes: Moniliales) on stored-grain beetles. J. Econ. Entomol. 94(2):367-372.
- Quintela, E.D. and C.W. McCoy. 1997a. Pathogenicity enhancement of *Metarhizium anisopliae* and *Beauveria bassiana* to first instars of *Diaprepes abbreviatus* (Coleoptera: Curculionidae) with sublethal doses of imidacloprid. Env. Entomol. 26(5): 1173-1182.
- Quintela, E.D. and C.W. McCoy. 1997b. Effects of imidacloprid on development, mobility, and survival of first instars of *Diaprepes abbreviatus* (Coleoptera: Curculionidae). J. Econ. Entomol. 90: 988-995.
- SAS Institute. 2003. User's Guide, Version 9.1. SAS Institute, Cary, NC.
- Thompson, S.R. and R.L. Brandenburg. 2004. A modified pool design for collecting adult mole crickets (Orthoptera: Gryllotalpidae). Fla. Entomol. 87(4): 582-584.
- Thompson, S.R. and R.L. Brandenburg. 2005. Tunneling responses of mole crickets (Orthoptera: Gryllotalpidae) to the entomopathogenic fungus, *Beauveria bassiana*. Env. Entomol. 34(1): 140-147.
- Thompson, S.R., Brandenburg, R.L., and J.J. Arends. 2005. Conidial viability and pathogenicity of *Beauveria bassiana* (Balsamo) Vuillemin for mole cricket (Orthoptera: Gryllotalpidae) control in turfgrass. Int. Turf. Soc. Res. J. 10: 784-792.
- Ulagaraj, S.M. 1976. Sound production in mole crickets (Orthoptera: Gryllotalpidae: *Scapteriscus*). Ann. Entomol. Soc. Am. 69: 299-306.

- Ulagaraj, S.M. and T.J. Walker. 1973. Phonotaxis of crickets in flight: attraction of male and female crickets to male calling songs. *Science*. 182: 1278-1279.
- Villani, M.G., L.L. Allee, L. Preston-Wilsey, N. Consolie, Y. Xia, and R.L. Brandenburg. 2002. Use of radiography and tunnel castings for observing mole cricket (Orthoptera: Gryllotalpidae) behavior in soil. *Am. Entomol.* 48: 42-50.
- Walker, T.J. and D. Ngo. 1982. Mole crickets and pasture grasses: damage by *Scapteriscus vicinus*, but not by *S. acletus* (Orthoptera: Gryllotalpidae). *Fla. Entomol.* 65: 105-110.
- Xia, Y., P.T. Hertl, and R.L. Brandenburg. 2000. Surface and subsurface application of *Beauveria bassiana* for controlling mole crickets (Orthoptera: Gryllotalpidae) in golf courses. *J. Agric. Urban Entomol.* 17: 177-189.
- Xu, Q.F. 1988. Some problems about study and application of *Beauveria bassiana* against agricultural and forest pests in China, pp. 1-9. *In* Y.W. Li, Z.Z. Li, Z.Q. Liang, J.W. Wu, Z.K. Wu, and Q.F. Xu (eds.), *Study and Application of Entomogenous Fungi in China*, vol. 1. Academic Periodical Press, Beijing, China.

Table 1. Mean mole cricket survival time (days) for 2004 and 2005 topical bioassays and mean survival time averaged over both years.

Treatment	2004	2005	Mean
Water control	26.10	26.70	26.40a
Clay control	26.60	24.40	25.50a
DE	26.85	22.05	24.45a
Imidacloprid	20.85	21.05	20.95abcd
Strain 5977 – 10 ⁸ conidia/cricket	8.55	13.25	10.90e
Strain 3622 – 10 ⁸ conidia/cricket	8.00	16.00	12.00e
Strain GHA – 10 ⁸ conidia/cricket	4.05	15.80	9.93e
Strain 5977 – 10 ⁷ conidia/cricket	22.00	22.60	22.30abc
Strain 3622 – 10 ⁷ conidia/cricket	23.95	22.60	23.275ab
Strain GHA – 10 ⁷ conidia/cricket	23.95	12.70	18.33abcde
5977 + DE	14.80	16.10	15.45bcde
3622 + DE	13.70	13.25	13.48de
GHA + DE	13.55	15.80	14.68cde
5977 + Imidacloprid	19.25	21.90	20.58abcd
3622 + Imidacloprid	13.20	18.75	15.98bcde
GHA + Imidacloprid	10.05	19.35	14.70cde

Means followed by the same letter in each column are not significantly different

(LSD t-test, $P = 0.05$).

Table 2. Statistical values for topical bioassay synergy tests (day 28 mortality) involving *B. bassiana* and diatomaceous earth or imidacloprid (N=8).

Combination Treatment	Estimate ¹	Standard Error	t value	<i>P</i>
Strain 5977 + DE	0.1875	0.0665	2.82	0.0059
Strain 3622 + DE	0.2500	0.0665	3.76	0.0003
Strain GHA + DE	0.1250	0.0665	1.88	0.0633
Strain 5977 + Imid.	-0.0125	0.0665	-0.19	0.8541
Strain 3622 + Imid.	0.1000	0.0665	1.50	0.1361
Strain GHA + Imid.	0.0125	0.0665	0.19	0.8514

¹Estimate = ((*B. bassiana* + DE/Imid) – *B. bassiana*) – (DE/Imid – Clay) using 28 DAT % mortality values

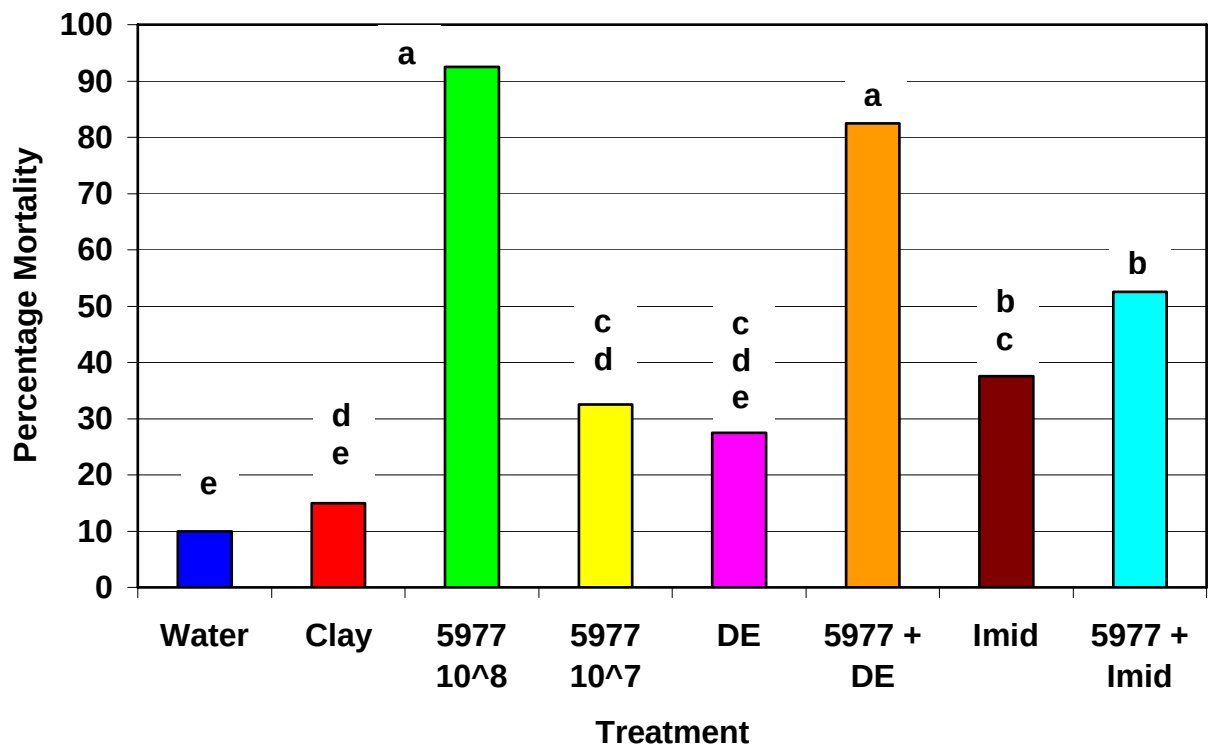


Fig. 1. Mean percentage mole cricket mortality at 28 DAT for *B. bassiana* strain 5977 treatments. Means followed by the same letter are not significantly different (LSD t-test, $P = 0.05$, SAS Institute, 2003).

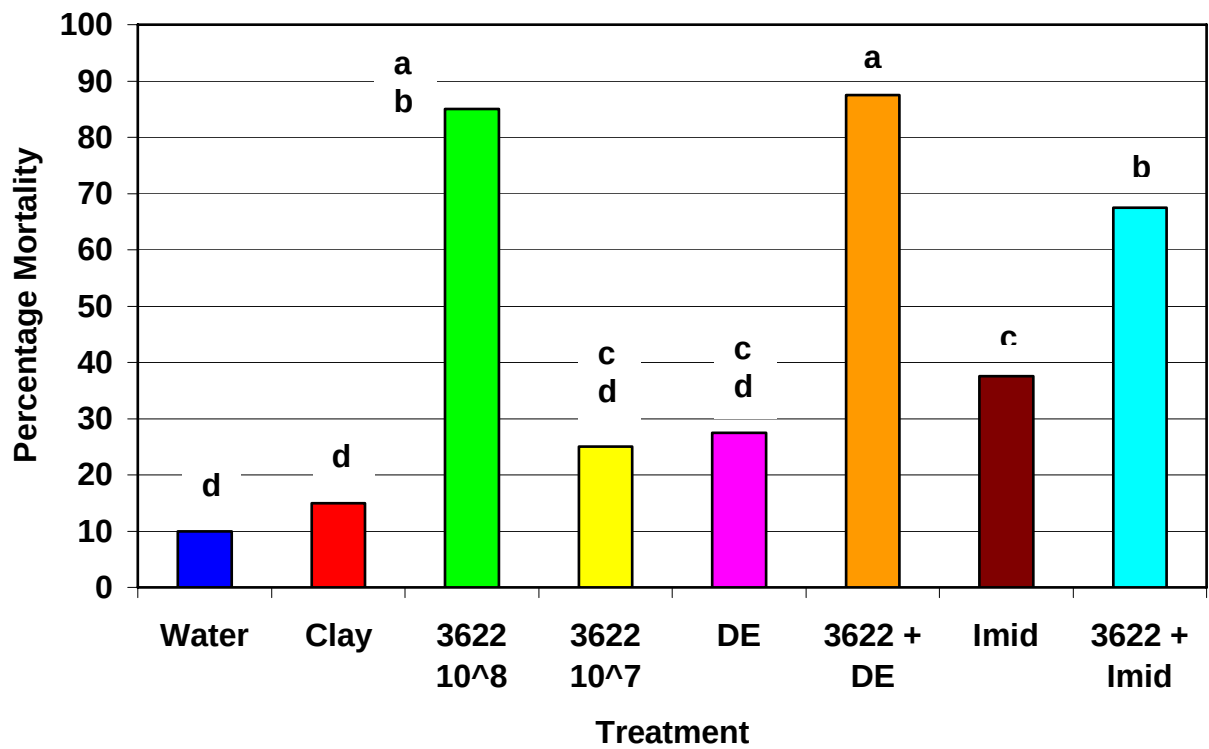


Fig. 2. Mean percentage mole cricket mortality at 28 DAT for *B. bassiana* strain 3622 treatments. Means followed by the same letter are not significantly different (LSD t-test, $P = 0.05$, SAS Institute, 2003).

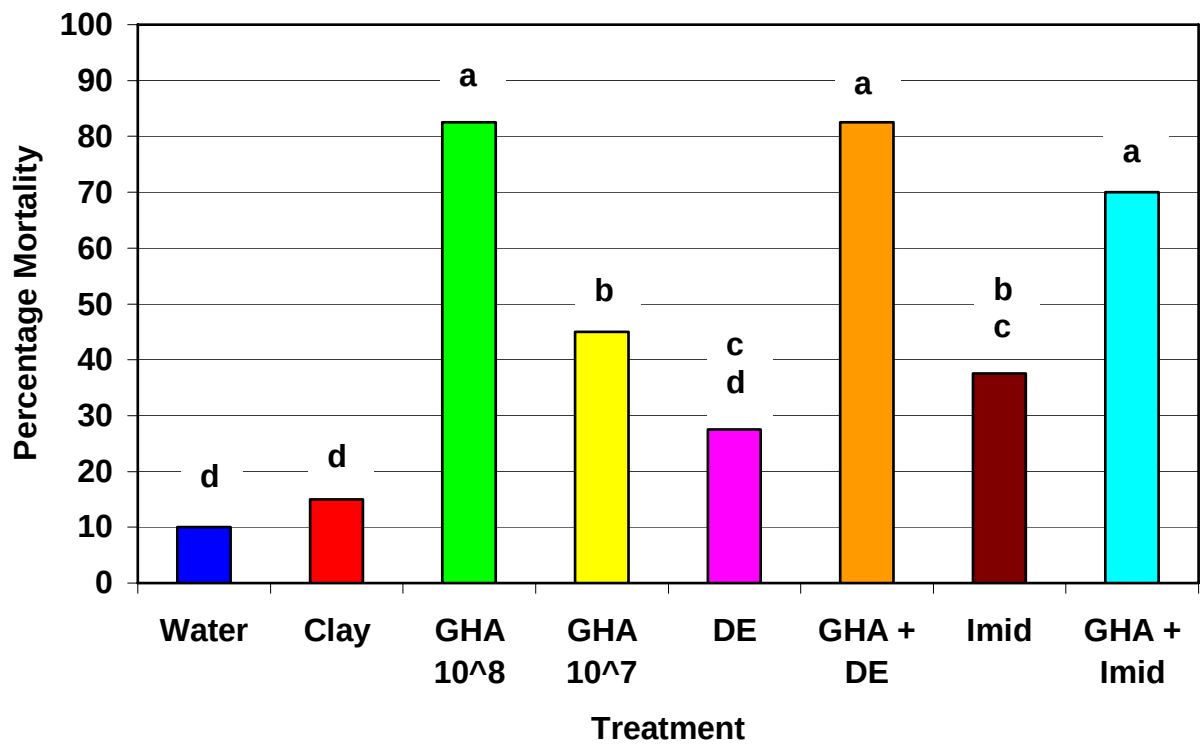


Fig. 3. Mean percentage mole cricket mortality at 28 DAT for *B. bassiana* strain GHA treatments. Means followed by the same letter are not significantly different (LSD t-test, $P = 0.05$, SAS Institute, 2003).

Florida Entomologist

Scientific Notes

Sarah Rachel Thompson

North Carolina State University

Department of Entomology

Box 7613

Raleigh, NC 27695-7613

Phone: (919) 513-2209

Fax: (919) 515-7746

sarah_thompson@ncsu.edu

**A TECHNIQUE TO MONITOR INSECT BEHAVIOR UTILIZING
INFRARED EMITTERS AND DETECTORS**

SARAH R. THOMPSON¹, RICK L. BRANDENBURG¹, GARY T. ROBERSON²,
and BARRY LINEBERGER²

¹Department of Entomology, North Carolina State University, Box 7613, Raleigh, NC

27695

and ²Department of Biological and Agricultural Engineering, North Carolina State

University, Box 7625, Raleigh, NC 27695

Monitoring insect movement is often necessary when examining behavioral responses to various stimuli, control agents, or repellents. Behavioral changes in the presence of entomopathogenic fungi have been documented for many insects, including mole crickets (Villani et al. 2002, Thompson and Brandenburg 2005). Due to the subterranean nature of these pests, creative techniques, such as radiographs and tunnel castings (Villani et al. 2002), have been employed to examine how crickets respond when placed in close proximity to repellent substances. In order to elucidate the mechanism of pathogen detection and avoidance, we wished to determine whether physical contact with the fungal conidia was necessary to initiate the behavioral response. Repellency away from the fungus without direct contact with the conidia suggests a chemosensory mechanism of detection.

One technique for monitoring insect movement is the use of photosensors, which are typically constructed out of an infrared emitter and its corresponding detector. Photosensors are placed in areas where it is necessary to monitor the presence of the insect. Photosensors have been used to examine the behaviors of ants (Sharma et al. 2004), blow flies (Cymborowski et al. 1996, Cymborowski 2003), and *Drosophila melanogaster* (Sheeba et al. 2001, Sheeba et al. 2002). We developed a behavioral assay chamber that utilizes two sets of photosensors to monitor mole cricket movement into fungi treated or untreated sand. This design allowed us to verify repellent behaviors towards specific fungal strains and determine whether the mole crickets require contact or close proximity to conidia in order to detect and avoid them (Thompson et al. 2006).

Treatment Containers. All behavioral tests involved a two-container setup that was constructed out of two 2.25L plastic food-storage containers (Ziploc[®] Large Rectangle

Snap 'n Seal Lids, SC Johnson and Son, Inc., Racine, WI). The containers were connected to one another via two 10-cm pieces of clear plastic tubing (1.59 cm outside diameter, 1.27 cm inside diameter, Watts[®] Clear Vinyl Tubing) that were attached in the center with a T-shaped plastic tube connector (Fig. 1). Each piece of tubing was attached to its respective container with hot glue at a circular opening made in the center of the longer side (approximately 13 cm from each end and 2.5 cm from the top). A 12-cm piece of tubing (entrance tube) was attached perpendicularly to the other two tubes with the “T” connector (Fig. 1). Both containers were filled with 1320g of 7% moisture Wando fine sand, either treated with the fungus or left untreated. This design allowed a mole cricket to be introduced into the center tube and choose which container to enter once it reached the top of the “T”.

Photosensors. A sensor was used to detect the passage of the cricket in the tubing and provide a means to record the event (the blocking of the infrared beam) in a data file. The sensor requirements were that they must be non-invasive, non-contact, and non-inhibitory to not promote or discourage cricket movement in any given portion of the tubing. A 1.8 cm diameter hole was bored with a milling machine into a 3.5 x 3.5 cm polyvinyl chloride block to slip around the tubing. The sensor circuit was constructed from an infrared emitter and detector combination (Part # 276-142, Radio Shack Corporation, Fort Worth, TX) that produces an infrared source in the 940 nm wavelength. Each infrared emitter was inserted into one side of the block and the detector was put in to the opposite side. Supply voltage is delivered to the emitter and a resistor in the same series limits the current flow (Fig. 2). The same voltage is provided to the detector (Fig. 2). When the infrared signal between the emitter and detector is interrupted by the passage of a cricket, the detector

responds by closing an internal switch thus allowing an event signal voltage to be recorded. The resistor in series with the detector pulls the voltage to zero when the detector is not registering an event. A regulated power supply was constructed to provide supply voltage to the sensor circuit at the voltage level required by the monitoring system. In total, 24 sensors were constructed.

Software. The data acquisition hardware for the experiment consisted of a desktop computer (Pentium 4 processor, 3.0 GHz, Windows XP) and an USB-based digital I/O data acquisition (daq) module (Model# PMD-1024LS, Measurement Computing Corp., Middleboro, MA). The 24 infrared photosensors were connected to the 24 digital inputs of the daq device. In order to shield against false positives in the data resulting from spurious voltage spikes, the circuit was constructed so that the signal output would only result when a mole cricket blocked the infrared beam. We created a program to periodically monitor the state of each sensor and to record the elapsed time when the state changes using LabVIEW™ programming language (National Instruments Corp., Austin, TX).

LabVIEW™ is a graphical programming language that uses icons, symbols, and diagrams rather than the text-based commands and functions of conventional programming languages. It was developed specifically to leverage the power, speed, and versatility of a personal computer coupled with relatively inexpensive sensor elements to create the functional equivalent of sophisticated laboratory instruments in software rather than in hardware. Thus, programs created in LabVIEW™ are dubbed “virtual instruments” (VI).

A LabVIEW™ VI consists of two windows or panels. The front panel, named Bug VI (Fig. 3), contains the controls and indicators comprising the user interface for the 12 experimental units that run simultaneously. An array of 24 led indicators show the current

state of each photosensor and light up each time a sensor is blocked by a cricket. The “start times” and elapsed “time” for each of the 12 individual experimental units are also shown in their respective indicator arrays. The “count” indicator shows the current value of the loop iteration terminal. A series of Boolean control buttons labeled “bug#1” through “bug#12” are used to set the start times for each cell when the cricket is first released into the entrance tube. The “stop” button halts program execution at the completion of the current iteration and the “reset all” button resets all start times to the default value so that the timers may be restarted without exiting.

The underlying program is constructed on the wiring panel (Fig. 4). The primary feature of this particular VI is the “while” loop containing code to read the sensor state and record the timing data as required (Fig. 5). A second “while” loop operating independently sets the start times for each cell when the operator sets the “bug#X” control (Fig. 6). Data is passed between the two “while” loops using local variables in order to prevent unwanted dataflow dependencies from interfering with proper execution sequencing. Other ancillary features outside the main “while” loop execute only at program initiation and serve to set initial values for the controls and indicators and to write column headings for the output data file.

The “while” loop executes all of the code it contains and then checks to see if the “stop” button has been pressed. If it has, then program execution stops. If not, the “while” loop performs another iteration. On each iteration, the 24 digital inputs from the photosensors are read and the current state is compared to the previous state. If any one of the states has changed, then a true value selects the true case of the if-then case structure (Fig. 7) and the current time and state values are written to the output data file. If there is no

change, then the “while” loop execution continues without data being written to the file. For this VI, a timing feature was included which pauses program execution for 1000 ms (1 sec) before resuming. Thus the count indicator is effectively a timer whose value represents the total elapsed time since the VI was initiated. In the output file, data are recorded as a 1 or –1 for every time that the sensor is blocked and then unblocked, respectively (Fig. 8). For each pair of sensors (one behavioral test unit), these values are logged relative to the particular time that the “bug#X” control is initiated. Since the software was written to log the particular time that any of the sensors are triggered, zero values for a particular pair of sensors indicate that an insect in another test unit has triggered a sensor.

SUMMARY

Photosensors constructed with infrared emitters and detectors were used to monitor mole cricket avoidance behaviors in association with entomopathogenic fungi. The sensors were non-invasive, non-contact, and non-inhibitory and therefore minimized impact on behaviors. Data were collected with a pc-compatible data acquisition module and logged to an output data file using custom-written software in the LabVIEW™ programming language. This technique offers an inexpensive, yet sophisticated, solution for monitoring insect behaviors without disruption.

REFERENCES CITED

- CYMBOROWSKI, B. 2003. Effects of 5,7-dihydroxytryptamine (5,7-DHT) on circadian locomotor activity of the blow fly, *Calliphora vicina*. J. Insect Sci. 3: 14-22.
- CYMBOROWSKI, B., S.F. HONG, H.G. MCWATTERS, and D.S. SAUNDERS. 1996. S-antigen antibody partially blocks entrainment and the effect of constant light on the circadian rhythm of locomotor activity in the adult blow fly, *Calliphora vicina*. J. Biol. Rhythms. 11: 68-74.
- SHARMA, V.K. 2003. A simple computer-aided device for monitoring activity of small mammals and insects. Biol. Rhythm Res. 34(1): 3-12.
- SHARMA, V.K., S.R. LONE, and A. GOEL. 2004. Clocks for sex: loss of circadian rhythms in ants after mating? Naturwissenschaften. 91: 334-337.
- SHEEBA, V., M.K. CHANDRASHEKARAN, A. JOSHI, and V. K. SHARMA. 2001. A case for multiple oscillators controlling different circadian rhythms in *Drosophila melanogaster*. J. Insect Physiol. 47: 1217-1225.
- SHEEBA, V., M.K. CHANDRASHEKARAN, A. JOSHI, and V. K. SHARMA. 2002. Developmental plasticity of the locomotor activity rhythm of *Drosophila melanogaster*. J. Insect Physiol. 48: 25-32.
- THOMPSON, S.R. and R.L. BRANDENBURG. 2005. Tunneling responses of mole crickets (Orthoptera: Gryllotalpidae) to the entomopathogenic fungus, *Beauveria bassiana*. Env. Entomol. 34(1): 140-147.
- THOMPSON, S.R., R.L. BRANDENBURG, and G.T. ROBERSON. 2006. Mechanism of entomopathogenic fungi detection and avoidance by mole crickets (Orthoptera: Gryllotalpidae). Env. Ent. In prep.

VILLANI, M.G., L.L. ALLEE, L. PRESTON-WILSEY, N. CONSOLIE, Y. XIA, and R.L.

BRANDENBURG. 2002. Use of radiography and tunnel castings for observing mole cricket (Orthoptera: Gryllotalpidae) behavior in soil. *Am. Entomol.* 48: 42-50.

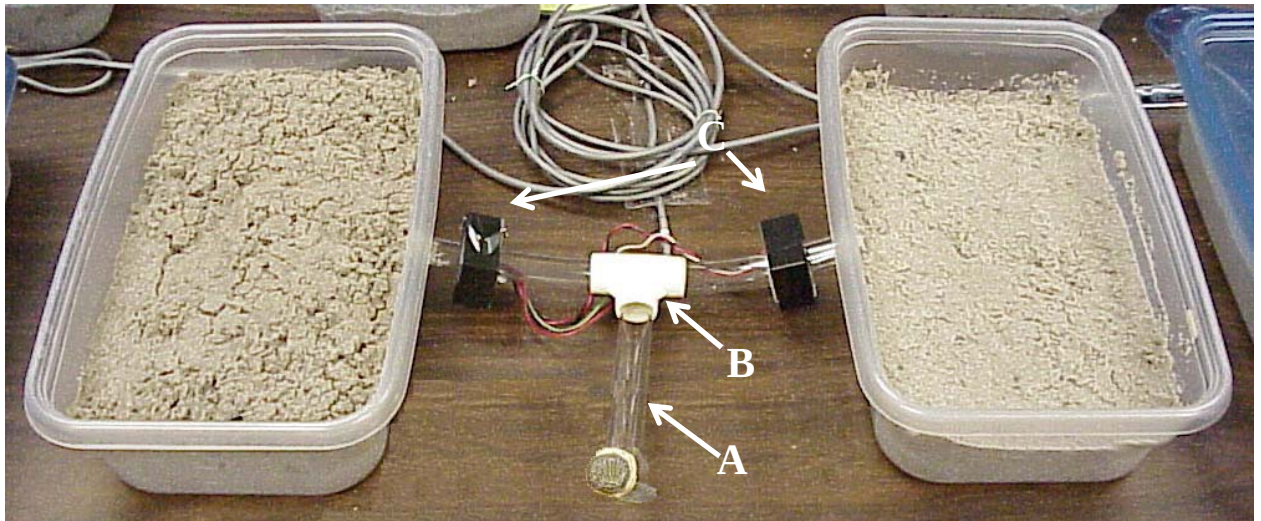


Fig. 1. Sample behavioral test unit, showing entrance tube (A), “T” connector (B), and photosensors (C)

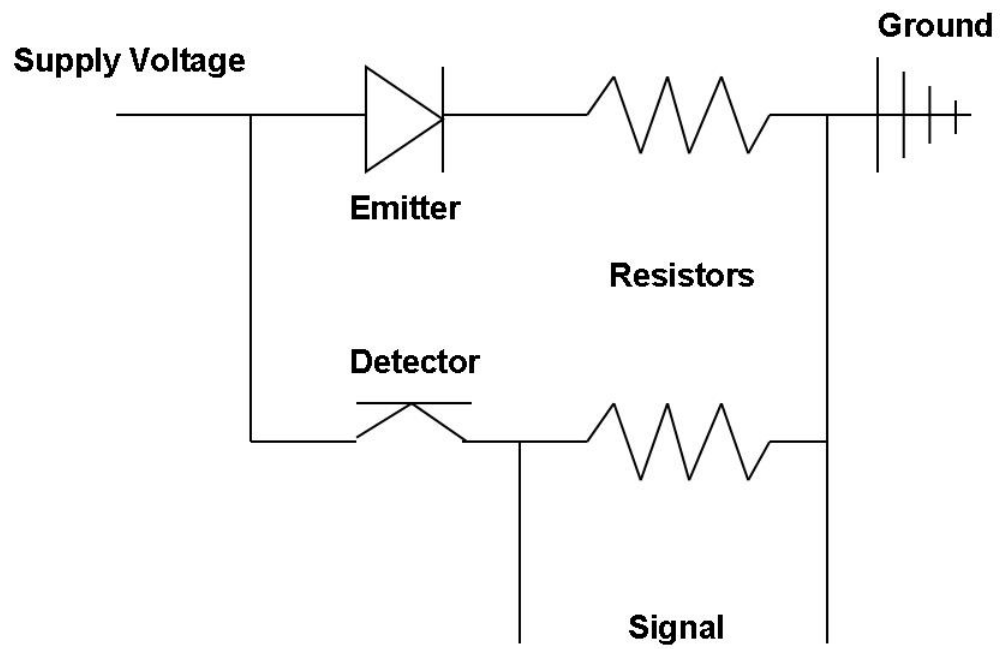


Fig. 2. Sensor circuit illustrating supply voltage, infrared emitter and detector, resistors, and signal output

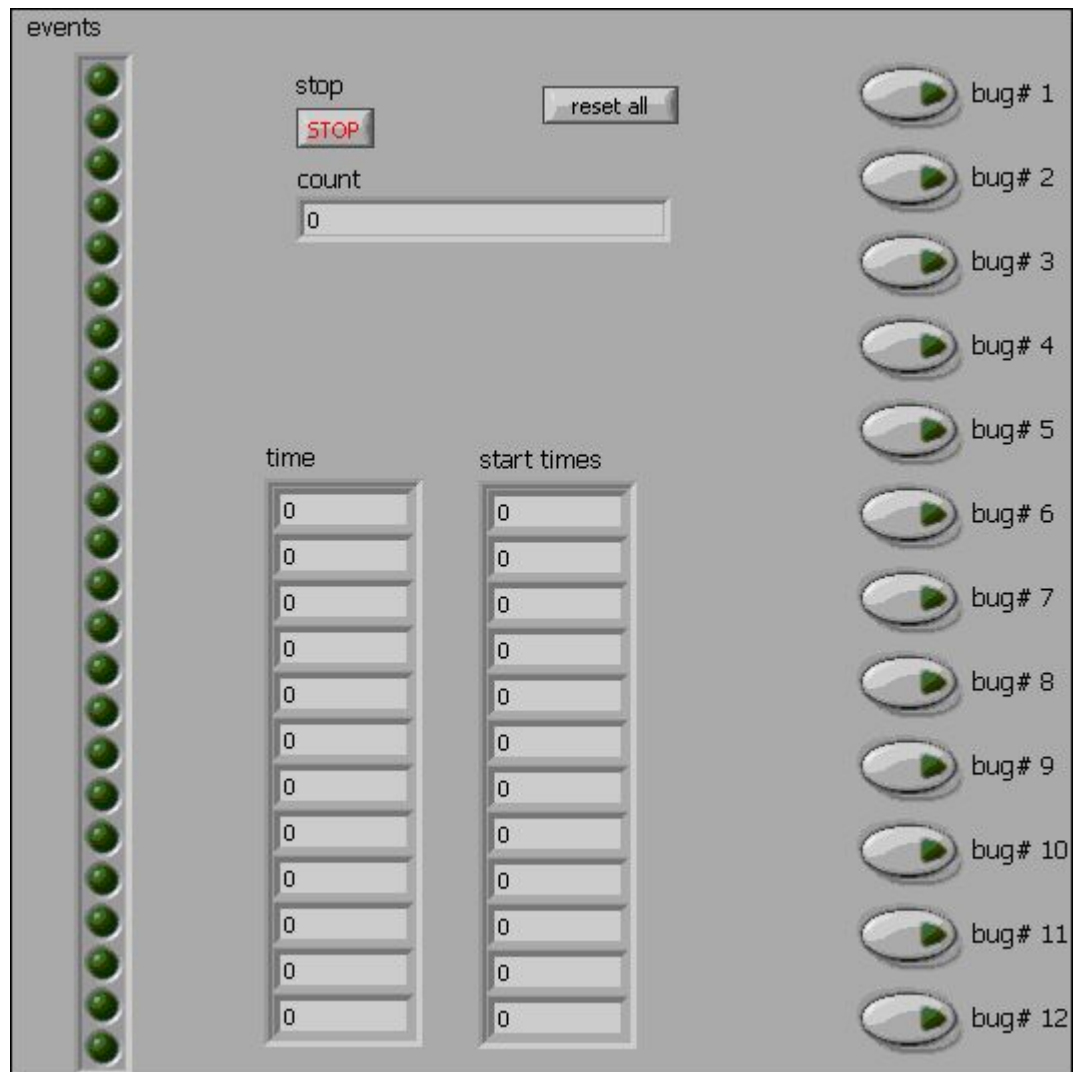


Fig. 3. Bug VI front panel (i.e. user interface) showing LED indicators for each event, control buttons for Bugs #1-12, start and elapsed times for each bug, and stop and reset buttons

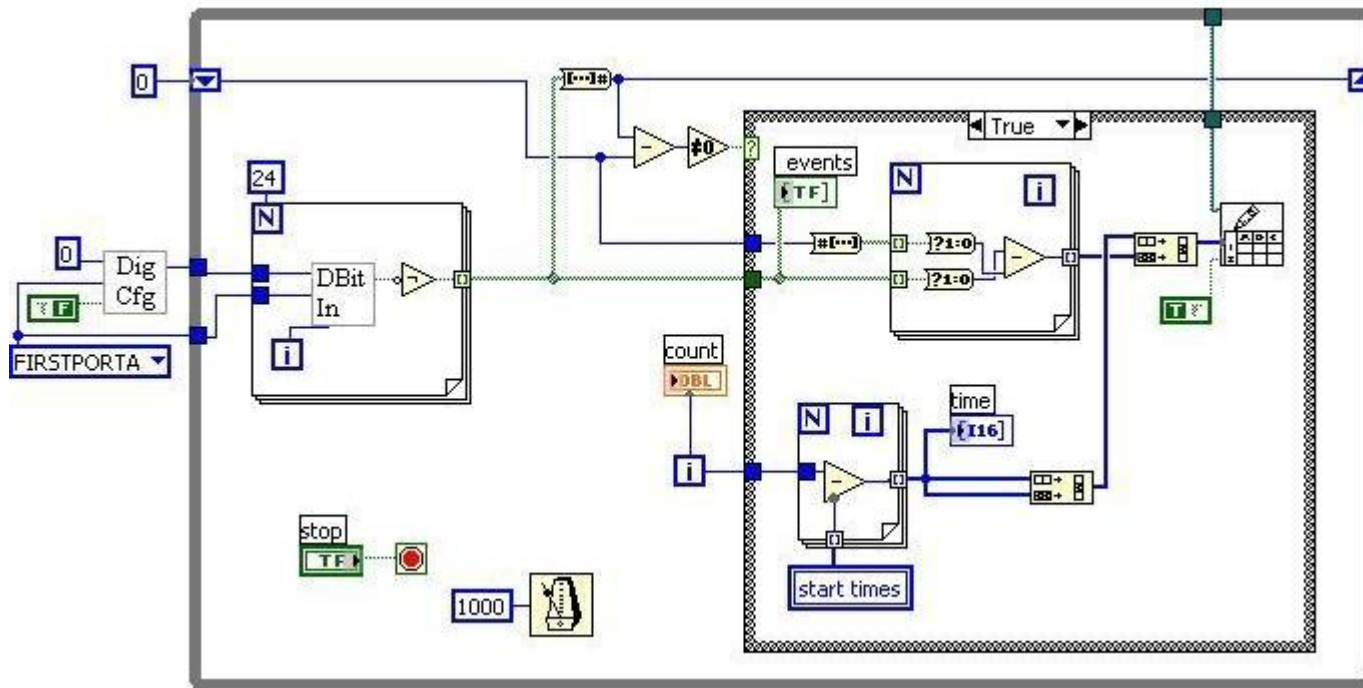


Fig. 5. Main “while” loop

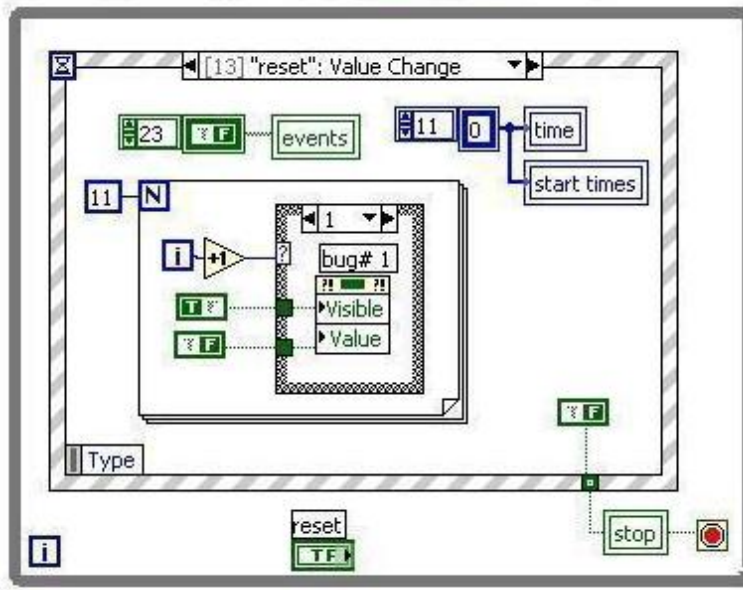


Fig. 6. Secondary “while” loop



Bug Test started at: 4/14/20054:50 PM			
time1	sensor1	time2	sensor2
45	0	45	1
46	0	46	-1
108	0	108	0
121	0	121	0
224	0	224	1
236	0	236	0
239	0	239	0
247	0	247	0
268	0	268	0
342	0	342	0
346	0	346	0
349	0	349	0
433	0	433	0
589	0	589	-1
592	0	592	1
609	0	609	0
1046	0	1046	-1
1154	0	1154	0
1155	0	1155	0
1403	0	1403	0
1419	0	1419	0
1447	1	1447	0
1488	-1	1488	0
1518	1	1518	0
1550	-1	1550	0
1566	0	1566	0
1610	0	1610	0
1611	0	1611	0
1615	0	1615	0
1616	0	1616	0
1618	0	1618	0
1634	1	1634	0
1637	-1	1637	0
1656	0	1656	1
1661	0	1661	-1
1697	0	1697	0
1711	0	1711	1
1713	0	1713	-1

Fig. 8. Example of data spreadsheet that shows the time associated with each occurrence of a sensor being blocked (1) and then unblocked (-1).

Environmental Entomology

Behavior

Thompson et al.: Mechanism of mole cricket
fungal avoidance

Sarah Rachel Thompson

North Carolina State University

Department of Entomology

Box 7613

Raleigh, NC 27695-7613

Phone: (919) 513-2209

Fax: (919) 515-7746

sarah_thompson@ncsu.edu

**MECHANISM OF ENTOMOPATHOGENIC FUNGI DETECTION AND
AVOIDANCE BY MOLE CRICKETS (ORTHOPTERA: GRYLLOTALPIDAE)**

SARAH R. THOMPSON¹, RICK L. BRANDENBURG¹, and GARY T. ROBERSON²

¹Department of Entomology, North Carolina State University, Box 7613, Raleigh, NC

27695

and ²Department of Biological and Agricultural Engineering, North Carolina State

University, Box 7625, Raleigh, NC 27695

Abstract

A chamber to monitor mole cricket behavior was designed utilizing two different soil-filled containers and photosensors constructed from infrared emitters and detectors. Mole crickets were introduced into a center tube that allowed them to choose whether to enter and tunnel in *Beauveria bassiana* treated soil or untreated soil. Each time the cricket passed through the photosensor located near the entrance of soil-filled containers, the infrared light was blocked and the exact moment that this occurred was logged onto a computer using custom-written software. The behaviors examined included the first photosensor trigger, total number of sensor triggers, presence of tunneling, and final location of the cricket after 18 h. These behaviors were analyzed to discern differences in mole cricket behavior in the presence of different treatments and to elucidate the mechanism that mole crickets use to detect pathogens. Five different strains of *B. bassiana* were tested in the first study; the second test involved the formulated product of one strain, the components of that product separately (conidia and carrier), and bifenthrin. In the first test, there were no differences between the first sensor trigger and total number of triggers, indicating the mole crickets are not capable of detecting *B. bassiana* at a distance greater than 5 cm. There were strain-specific differences in the occurrence of tunneling and remaining in containers filled with *B. bassiana* treated sand. This finding suggests that mole crickets can detect and choose to avoid contact with fungal pathogens, but must come into close proximity with them for recognition. There were no differences between treatments in the second study. Since the first test involved *Scapteriscus borellii* and the second used *S. vicinus*, results suggest a more sensitive chemosensory recognition system for southern mole crickets.

KEY WORDS: infrared emitters and detectors, *Beauveria bassiana*, *Scapteriscus borellii*, *S. vicinus*, avoidance

Introduction

Areas of managed turfgrass have grown significantly over the past two decades, and along with that growth has come an increased focus on control of turfgrass pests. Mole crickets are considered to be one of the most significant groups of turf and pasture grass pests in the southeastern U.S. (Walker and Ngo 1982) due to the substantial damage they cause. The tawny mole cricket, *Scapteriscus vicinus* Scudder, feeds almost entirely on plant material, while *S. borellii* Giglio-Tos, the southern mole cricket, is predominantly predatory (Ulagaraj 1975, Taylor 1979, Matheny 1981). The feeding on the roots by *S. vicinus* can make its damage more substantial than *S. borellii*, but both species cause desiccation of the root system through the production of extensive underground and surface tunnels (Villani et al. 2002). Over the course of the summer as the nymphs develop and increase their feeding, damage appears as the thinning of turfgrass stand with eventual total turf loss possible (Frank and Parkman 1999). Total loss of turfgrass can lead to an invasion by opportunistic weeds and the need for reestablishment. Even small populations of mole crickets can be significant if they attract predators such as armadillos, raccoons, or birds, which cause serious damage in attempts to forage for food (Frank and Parkman 1999).

Due to their subterranean nature, chemical control of mole crickets is often difficult to accomplish without the use of higher insecticide rates or repeated applications (Xia et al. 2000). Additionally, because of the cosmetic nature of turfgrass, it is difficult to determine threshold levels and establish acceptable levels of control; these vary by use, location, and clientele expectations. Some of the issues that are associated with the use of synthetic

insecticides in turfgrass include potential impacts on surface and groundwater, non-target wildlife, human exposure, and persistence and degradation in the environment (Racke 2000). There is societal concern over the use of chemical insecticides in turfgrass that is in close proximity to environmentally-sensitive areas such as irrigation lakes, wildlife habitats, or residential homes (Villani et al. 2002, Thompson and Brandenburg 2005). Because of these concerns, it has become critical to explore alternative methods of control that pose fewer risks to the environment.

Biological control agents, such as pathogenic fungi, are an alternative option for mole cricket control in turfgrass (Brandenburg and Villani 1995). Unlike entomopathogenic nematodes, fungal conidia do not require entry through a bodily opening in order to infect a pest insect. The conidia attach to the insect cuticle and germinate, penetrating into the hemocoel, and cause mortality through the depletion of hemolymph nutrients or production of toxic fungal metabolites (Jaronski and Goettel 1997). *Beauveria bassiana* (Balsamo) Vuillemin is one of the most widely researched and efficacious species of entomopathogenic fungi, with pathogenicity shown to over 200 different species of insects (Feng et al. 1994). Only a few field studies using this naturally-occurring soil fungus have been conducted on mole crickets, but data have failed to show consistent levels of acceptable control (Hertl and Brandenburg 1998, Harris 1999, Xia et al. 2000). Strain specific pathogenicity has been shown towards mole crickets, with strains isolated from Orthoptera causing the most mortality in the shortest amount of time after exposure (Thompson et al. 2005).

One possible explanation for the lack of efficacy observed in the field studies despite proven pathogenicity in the laboratory is mole cricket repellency to *B. bassiana* conidia. Villani et al. (2002) and Thompson and Brandenburg (2005) observed changes in mole

cricket tunneling behaviors in order to avoid contact with *B. bassiana* conidia. It is believed that crickets exhibit these avoidance behaviors in order to prevent contact with the conidia until they are no longer viable and infective. The mechanism of detection is unknown, but is hypothesized to be chemosensory. Our objectives were to determine if physical contact with *B. bassiana* conidia evokes mole cricket avoidance behaviors and to test for differences between the repellent behaviors of *S. vicinus* and *S. borellii*. Photosensors utilizing infrared emitters and detectors have been used to monitor the behaviors of ants (Sharma et al. 2004), blow flies (Cymborowski et al. 1996, Cymborowski 2003), and *Drosophila melanogaster* (Sheeba et al. 2001, Sheeba et al. 2002). We developed a behavioral assay chamber that utilizes two sets of photosensors (each made from one infrared emitter and its corresponding detector) to monitor mole cricket movement into *B. bassiana* treated or untreated sand (Thompson et al. 2006 in prep.). This design allowed us to verify repellent behaviors towards specific *B. bassiana* strains and determine whether the mole crickets require either contact or close proximity to conidia in order to detect and avoid them. We hypothesize that mole crickets use chemosensory cues to detect pathogenic fungi and that southern mole crickets are more sensitive to these cues than tawny mole crickets.

Materials and Methods

Propagation of Fungal Isolates. Four of the strains of *B. bassiana* used in this study, MC, DB-2, 5977, and 3622 were propagated on a solid substrate and formulated in an emulsifiable oil carrier by JABB of the Carolinas, Inc. (Pine Level, NC). Strain MC was isolated by S.R. Thompson from *S. vicinus* in May 2004 in North Carolina. Strains 5977 and 3622 were obtained as cultures from the ARS Collection of Entomopathogenic Fungal Cultures (Ithaca, NY). Strain 5977 is IMI (331275) isolate and its origination is identified as

Orthoptera with no specific location. J.L. Stimac isolated strain 3622 in Florida from *S. vicinus*. These three strains were chosen since all originate from orthopteran hosts and previous research indicates that strains isolated from a close relative of the target host are the most pathogenic (Thompson et al. 2005). DB-2 was originally obtained from darkling beetles in poultry houses in North Carolina (Apuya et al. 1994). The fifth strain of *B. bassiana*, strain GHA, was obtained from Emerald Bioagriculture Corporation (Butte, MT) as conidia and a formulated product (BotaniGard®). The other treatment used in these studies for comparison was the commercially available turfgrass insecticide, bifenthrin (Talstar® Flowable Insecticide/Miticide, FMC, Philadelphia, PA).

Collection of Mole Crickets. For the first test, adult *S. borellii* were collected from four golf courses (The Lakes Country Club in Boiling Springs Lakes, Sandpiper Bay Golf Course in Sunset Beach, Olde Fort Golf Club in Wilmington, and The Duck Golf Club, also in Wilmington) in New Hanover and Brunswick Counties, NC during the months of March through May 2005. Crickets were attracted to electronic callers (Ulagaraj and Walker 1973) that mimic the male mole cricket calls, which they produce in the evenings during the spring months to attract females for mating (Ulagaraj 1976). The callers were suspended above a modified pool catch trap, which utilized two plastic wading pools, one placed on top of the other (Thompson and Brandenburg 2004). The pool on top had holes cut into it, through which the crickets fell after they flew to the callers, and the bottom pool was filled with moist sand to hold the crickets until they were harvested. Crickets were collected on a weekly basis during these three months. All crickets were held individually to prevent cannibalism and maintained in soil from The Lakes Country Club collection site (classified as Wando fine sand, HM = 0.36%, pH = 6.2, CEC = 4.5) at 10% moisture in a climate-

controlled laboratory on the campus of North Carolina State University (Raleigh, NC). The crickets were fed earthworms until the behavioral tests were performed.

For the second trial, nymphal *S. vicinus* mole crickets were collected in Aug and Sept 2005 from Sandpiper Bay Golf Course driving range. Crickets were flushed to the surface using a soapy water flush consisting of a 0.4% solution of Joy® brand liquid dishwashing detergent (Proctor and Gamble, Cincinnati, OH) in water (Short and Koehler 1979). Once the crickets reached the surface, they were rinsed in water to remove any soap residue and placed in plastic buckets filled with moist soil from The Lakes Country Club site. All crickets were held and maintained in large plastic containers filled to a depth of 45 cm with 10% moisture soil from the collection site. The crickets were kept in a greenhouse on the campus of North Carolina State University and allowed to feed on bermudagrass *Cynodon dactylon* (L.) sod. No crickets were used in behavioral studies until they had been maintained in the greenhouse for at least two weeks after the soapy water flush collection date.

Behavioral Studies. All behavioral tests involved a two-container setup that was constructed out of two 2.25L plastic food-storage containers (Ziploc® Large Rectangle Snap ‘n Seal Lids, SC Johnson and Son, Inc., Racine, WI). The containers were connected to one another via two 10-cm pieces of clear plastic tubing (Watts® Clear Vinyl Tubing) that were attached in the center with a T-shaped plastic tube connector (Fig. 1). Each piece of tubing was attached to its respective container with hot glue at a circular opening made in the center of the longer side (approximately 13 cm from each end and 2.5 cm from the top). A 12-cm piece of tubing (entrance tube) was attached perpendicularly to the other two tubes with the “T” connector (Fig. 1). Both containers were filled with 1320g of 7% moisture

Wando fine sand. This design allowed a mole cricket to be introduced into the center tube and choose which container to enter (treated or untreated) once it reached the top of the “T”.

Photosensors utilizing infrared emitters and detectors were placed 2.0 cm from the containers (Fig. 1) and monitored mole cricket entry into each container (Thompson et al. 2006 in prep.). A set of one infrared emitter and one detector was placed in opposite ends of a square plastic block that was bored out to allow passage of the clear tubing through it. Our use of infrared emitters and detectors was similar to Sharma (2003), but a different data acquisition system and software were used to collect the data on the computer. Data were compiled with a 24-port USB-based I/O digital data acquisition module (Model# PMD-1024LS, Measurement Computing Corp., Middleboro, MA). Custom-written software using the LABView™ programming language (National Instruments Corp., Austin, TX) logged data onto a desktop computer ((Dell Inc., Round Rock, TX) with an Intel® Pentium® 4 processor (Santa Clara, CA), 3.0 GHz and Microsoft® Windows XP (Redmond, WA)) for 18 h after the start of the test (Thompson et al. 2006 in prep.). Every time a mole cricket passed through a photosensor, the exact time that the infrared light was blocked was logged onto a spreadsheet on the computer. The software design allowed the user to click on an icon that corresponded to each behavioral container so that the time elapsed would be based on the exact time that each specific cricket entered the center tube (i.e. start time). This was important since the crickets were placed in the entrance tubes one at a time, and it typically took an average of ten minutes to put all crickets into their respective tubes. After the mole cricket was placed into the entrance tube, the end of the tube was sealed with a small piece of fiberglass window screen (New York Wire, Mt. Wolf, PA) secured with a rubber band. All tests were conducted under black landscaping plastic (Warp’s Carry-home® Coverall® 4

mil Plastic sheeting, Chicago, IL) to prevent irregular mole cricket behaviors due to light exposure and all windows in the test room were covered with the same plastic.

Trial I. In the spring of 2005, a study was conducted to evaluate the behavioral responses of southern mole crickets to various strains of *B. bassiana* conidia that were formulated in emulsifiable oil carriers. The six treatments included strains MC, DB-2, 5977, and 3622 (formulated in JABB's proprietary carrier), strain GHA (formulated in Emerald Bioagriculture's proprietary carrier), and an untreated control. Each replication consisted of one *B. bassiana* treated container and one untreated container (the control consisted of two untreated containers). 100 ml of liquid, composed of distilled water and 1.665×10^{10} conidia in the emulsifiable oil carrier, was added to each treated container and this resulted in the 7% soil moisture level. All untreated containers were composed of Wando fine sand and 100 ml of distilled water, so that soil type and moisture were consistent across all treatments. Since a 24-port data acquisition module was used, 24 photosensors could run simultaneously; each behavioral test utilized two photosensors (one each for the treated and untreated container) and this allowed for 12 replicates per night. For the first test, six treatments were evaluated in total with two replicates of each conducted per night. Tests were conducted on 20 different nights between 14 Apr 2005 and 30 May, for a total of 40 replications per treatment.

Trial II. A second study was conducted in the fall of 2005 with tawny mole crickets to test the repellency of the different components (carrier and conidia) compared to the formulated product and a conventional insecticide, bifenthrin. Strain MC was chosen for this test and an untreated control was also included. The same number of conidia was added to each container for the formulated treatment as for the unformulated conidia, and moisture

levels were consistent across all treatments. An equivalent amount of carrier was added to the formulated product and carrier only treatments. Bifenthrin applications were made at the rate of 1.0 oz/1000 ft², the higher label rate recommended for control of large mole cricket nymphs or adults. Since this follow-up test included 5 treatments, 20 ports of the data acquisition module were used, which still allowed for 2 replications of each treatment per night. Tests were conducted on 15 different nights between 18 Oct and 7 Nov, for a total of 30 replications per treatment.

For all tests, treatments were randomly assigned to containers each night, and the treated side alternated between the left and right containers to eliminate any mole cricket directional preferences. After the treated and untreated soils were added to each test container, plastic lids were placed on top of all containers and the crickets were introduced into the entrance tubes one at a time. 18 h after the start of the last treatment, the test was ended by stopping the software program on the computer (Thompson et al. 2006 in prep.) and the plastic covers were removed from all containers. Each container was checked for the presence of new surface tunneling and as the soil was emptied, the final location of each mole cricket was recorded. All containers, lids, and “T” connectors were sterilized in 10% bleach (6% sodium hypochlorite, The Clorox Company, Oakland, CA) solution before use and between replications to prevent contamination. All tubing was removed from the containers after each night’s tests and replaced with new pieces of tubing. Since the software logged the exact time that any of the photosensors was blocked, the following data was extracted from the spreadsheets: length of time before the first sensor trigger (and which sensor was triggered first) and the number of times each sensor was triggered.

Statistical Analysis. For the percentage of total triggers that occurred in treatment-associated photosensors, data were analyzed by factorial analysis of variance using the General Linear Models Procedure of SAS (PROC GLM, SAS Institute 2003). This procedure was also used to test for directional preferences indicated by a predominance of tunneling in one side or another of all chambers. For the percentage of first triggers that occurred on treatment sides, occurrence of tunneling in each container, and final location of the cricket, data were analyzed by examining the strength of association between the mole cricket behaviors and treatments with a 2-way contingency table and Likelihood Ratio χ^2 test ($P = 0.05$) (PROC FREQ, SAS Institute 2003). Comparisons between each treatment and the control were made by fitting the data to a generalized linear model, conducting an analysis of parameter estimates, and calculating χ^2 values for the explanatory variables (treatments) using PROC GENMOD (SAS Institute 2003).

Results and Discussion

Overall, there were no directional preferences exhibited by the crickets that indicate they prefer turning right or left into the tubing once reaching the top of the “T” connector. This was the case for the first trial ($F = 1.71$; $df = 1,429$; $P = 0.192$) involving southern mole crickets as well as Trial II ($F = 0.29$; $df = 1,261$; $P = 0.593$) with tawny mole crickets. Even though the treatments were randomly assigned to treatment containers each night, it was necessary to verify that results were not associated with any predisposed mole cricket directional preferences.

First photosensor trigger. Prior to conducting the studies, we believed that the mole crickets would be able to sense the *B. bassiana* treatments in the containers from the “T” connector (a distance of approximately 8.0 cm), and would avoid contact with the

conidia altogether by immediately entering the tube and corresponding container filled with untreated soil. A lack of preference or detection for one side or another would be indicated by mole crickets triggering photosensors on each side of the behavioral chamber equally (i.e. 50% for both sides of the chamber). It appears that the mole crickets cannot detect the conidia from this distance and which tube they choose to initially travel through is random, since there were no significant differences in the overall percentage of photosensors that are first triggered for treated and untreated containers. This was the case in the first study involving formulated *B. bassiana* strains ($\chi^2 = 2.00$; $df = 5$; $P = 0.850$) and southern mole crickets as well as the second trial with tawny mole crickets, which included other treatments such as bifenthrin and the carrier of the conidia ($\chi^2 = 0.54$; $df = 4$; $P = 0.970$) (Fig. 2). In Trial I, the photosensors associated with all five strains of *B. bassiana* were triggered at rates almost equivalent to the control-control treatment, which showed that both sensors were triggered first equally (52.63 and 47.37% for the left and right sides, respectively) (Fig. 2).

The same trend was exhibited in the second study, where both sides of all treatments were first triggered equally. The percentage occurrence of the treated container being triggered first was 50.00% for MC (formulated product), 56.67 % for MC conidia only, 50.00 % for carrier, and 53.33% for bifenthrin. These percentages were again similar to the observed ratios for the control containers, which were triggered first 48.28% and 51.72% of the time in the left and right sides, respectively. The consistency in results for these behaviors suggests that both species of mole crickets randomly turn right or left when given the choice to go in two different directions, and this behavior is not influenced by the presence of *B. bassiana* conidia or other insecticides that are more than 5 cm away. Because

mole crickets typically construct subterranean “Y” shaped tunnels (Villani et al. 2002), it is logical that their behavior, even in artificial situations, exploits the opportunities that exist with two routes of movement.

Number of photosensor triggers. Since we observed that mole crickets randomly turn right or left in the tubing regardless of the location of the treatment or control, it was necessary to examine how many times the photosensors were triggered. We initially believed that the only time that the mole cricket would block the infrared light would be when passing through the sensor in route to a sand-filled container. After the first night of testing, it was determined that there were often sensors triggered in instances when the mole cricket actually never entered the container (as evidenced by tunneling). The mole crickets were much more mobile in the tubes than expected, and were able to trigger sensors, back up, and then trigger again, often multiple times. Therefore, a significantly different number of photosensor triggers in any treatment in comparison to a control could indicate a change in behavior when in proximity to pathogens or other treatments in the soil. In order to make more accurate comparisons, data were transformed from total number of photosensor triggers to percentage of triggers (for the treatment and control containers) out of total triggers for that particular behavioral chamber.

The percentage of triggers that occurred at treatment-associated photosensors was not significantly different than the percentage of triggers that occurred on each side of the control chamber in the first study involving the 5 *B. bassiana* strains ($F = 0.63$; $df = 5,190$; $P = 0.676$) or the second study ($F = 0.84$; $df = 4,115$; $P = 0.501$). An equal number of photosensor triggers (50%) was expected for both sides of the control chamber, and this was the case in both tests. In Trial I, the left sensor of the control chamber had 44.95% of the

total number of triggers (Fig. 3) and in Trial II, the left sensor of the control chamber had 48.28% of the total number of triggers. Although smaller percentages of triggers occurred near the entrance to the treated soil for all five *B. bassiana* strains (Fig. 3), these numerical differences were not significant.

The same trend was observed in the second trial; the percentage occurrence of triggers at the treatment container was 33.38% for MC (formulated product), 35.30 % for MC conidia only, 38.51 % for carrier, and 45.99% for bifenthrin. These data indicate that detection and avoidance behaviors for both southern and tawny mole crickets do not require significantly more associations with pathogens in order to differentiate between untreated soil and soil containing control agents. In approximately 80- 90% of all containers in both tests, mole crickets triggered both sides of all treatment containers multiple times, rather than entering the center tube and quickly running down one side or another to enter the sand and only triggering one sensor once. This behavior indicates that the act of tunneling or remaining in a particular container is a triggered response and not a random choice made by the mole crickets.

Presence of tunneling. Because the soil surface in the containers was at a level just above the opening where the tubing connected, any tunneling by a mole cricket was readily observable. The presence of tunneling verified that the mole cricket had made physical contact with the soil and any treatment in it. There were significant differences in the presence of tunneling for the first trial ($\chi^2 = 13.67$; $df = 5$; $P = 0.0178$) (Fig. 4). There was significantly less occurrence of tunneling in sand treated with *B. bassiana* strains 3622 ($\chi^2 = 5.93$; $df = 1$; $P = 0.0149$), MC ($\chi^2 = 7.69$; $df = 1$; $P = 0.0055$), and DB-2 ($\chi^2 = 7.44$; $df = 1$; $P = 0.0064$) compared to the untreated control (Fig. 4). This behavior indicates that the S.

borellii adults are able to detect and avoid contact with conidia of certain strains of *B. bassiana*. Thompson and Brandenburg (2005) have also shown that mole crickets do not exhibit universal repellency behaviors towards all strains of entomopathogenic fungi, but instead responses are strain specific.

In the second test, there were no differences in the presence of tunneling for any of the treatments ($\chi^2 = 6.42$; $df = 4$; $P = 0.1701$). The percentage occurrence of tunneling in the treated container was 25.93% for MC (formulated product), 30.77 % for MC conidia only, 17.39 % for carrier, and 34.78% for bifenthrin. Since there were no differences between the control and any of the treatments in this study (relative to the occurrence of tunneling), it appears that there may be differences in the behaviors of the two species of mole crickets tested. *S. borellii* adults avoided contact with strain MC in the first study, while *S. vicinus* nymphs did not in the follow-up test; this suggests that southern mole crickets exhibit more extreme avoidance behaviors to *B. bassiana*. This supports the observations of Villani et al. (2002) in their behavioral assays involving both species of mole crickets and the entomopathogenic fungi *M. anisopliae* and *B. bassiana*. Differences in behaviors are logical given the biology of the two species. The predatory nature of southern mole crickets might suggest a more sensitive sensory system that facilitates the location of prey. Tawny mole crickets typically construct tunnels at the soil-root zone interface (Villani et al. 2002) and therefore, food is readily available in that location at all times.

Final location of cricket. There were a number of instances when mole crickets tunneled through the sand in a particular container, but eventually left that location to go into the other side. A reduced occurrence of mole crickets being found in containers filled with treated soil may suggest that the mole crickets avoid contact with the treatment in that

container. Similar to the other behaviors examined, a lack of repellency would be indicated by an equal percentage of crickets choosing to remain in either side of a chamber. The control-control treatment had 37.50% and 62.50% of the crickets in the left and right sides, respectively (Fig. 5). All five *B. bassiana* strains caused fewer southern mole crickets to remain in the treated soil rather than the corresponding untreated container (Fig. 5). There were significant differences between the percentage of southern crickets that were discovered in sand-filled containers treated with *B. bassiana* strains 3622 ($\chi^2 = 4.47$; $df = 1$; $P = 0.0344$), MC ($\chi^2 = 7.41$; $df = 1$; $P = 0.0065$), DB-2 ($\chi^2 = 6.18$; $df = 1$; $P = 0.0129$), and GHA ($\chi^2 = 4.23$; $df = 1$; $P = 0.0398$) compared to the untreated control (Fig. 5). Three out of four of these strains were also significant for the presence of tunneling. The discrepancy for strain GHA indicates that the southern mole crickets may not have been able to detect the spores as well as the other three strains, but still were repelled enough to avoid remaining in GHA-treated sand.

In the second study involving tawny mole crickets, there were no significant differences between any of the treatments ($\chi^2 = 7.47$; $df = 4$; $P = 0.1129$) in regards to the percentage occurrence of crickets tunneling in treated versus untreated sand. The percentage of tawny mole crickets found in containers filled with treated sand was 25.93% for the formulated MC product, 29.63% for MC conidia only, 17.39% for the carrier, and 42.31% for bifenthrin. All of these treatments were numerically less than the 48.28% and 51.72% of crickets found in the left and right sides of the control-control chamber, but the differences were not significant. Like the percentage occurrence of tunneling, these results suggest a difference in behaviors between southern and tawny mole crickets, with *S. borellii* exhibiting stronger avoidance responses.

In conclusion, these studies indicate that mole crickets are capable of detecting entomopathogenic fungi in the soil without making physical contact, but are not able to recognize the conidia at distances greater than 5 cm. This suggests a chemosensory mechanism of detection. The avoidance behaviors exhibited by southern mole crickets suggest that they will completely avoid tunneling in soil treated with *B. bassiana*. If total avoidance is not possible, the mole crickets will be repelled from *B. bassiana* treated soil and will not remain in that soil for long periods of time. It appears that southern mole crickets have more sensitive sensory perception since their avoidance responses were more extreme than those exhibited by tawny mole crickets towards the same strain of fungus. Results from these studies provide insight into why field studies using *B. bassiana* for mole cricket control have failed to provide significant long-term control. In areas with severe mole cricket infestations, the application of *B. bassiana* may provide a short period of reduced turfgrass damage due to the avoidance behaviors of the crickets. However, because contact between the host and agent is required for infection, the repellent nature of pathogenic conidia for mole crickets will likely reduce mortality and thus long-term efficiency of such treatments. Future research should focus on methods to minimize detection and avoidance of *B. bassiana* through the use of masking agents, baits, or other similar products. It would also be interesting to discern the differences in behavior between the two mole cricket species by performing electroantennagrams.

Acknowledgements

The authors gratefully acknowledge Dr. Cavell Brownie in the Department of Statistics, NCSU for her assistance with the statistical analysis of data. We are extremely thankful for the help of Phil Harris in the Department of Biological and Agricultural Engineering for constructing and wiring the photosensors. Special thanks to the superintendents/owners of all four golf courses for allowing the (loud) calling traps to function at those locations during the mole cricket collection periods. The reviews and suggestions of Drs. Clyde Sorenson and Jack Bacheler are greatly appreciated. Partial support for the completion of this research was provided by the Center for Turfgrass Environmental Research and Education in Raleigh, NC.

References Cited

- Apuya, L.C., S.M. Stringham, J.J. Arends, and W.M. Brooks. 1994. Types and prevalence of protozoan infections in darkling beetles from poultry houses in North Carolina. *J. Invertebr. Path.* 63: 255-259.
- Brandenburg, R.L. and M.G. Villani. 1995. Handbook of Turfgrass Pests. Entomological Society of America, Lanham, MD.
- Cymborowski, B. 2003. Effects of 5,7-dihydroxytryptamine (5,7-DHT) on circadian locomotor activity of the blow fly, *Calliphora vicina*. *J. Insect Sci.* 3: 14-22.
- Cymborowski, B., S.F. Hong, H.G. McWatters, and D.S. Saunders. 1996. S-antigen antibody partially blocks entrainment and the effect of constant light on the circadian rhythm of locomotor activity in the adult blow fly, *Calliphora vicina*. *J. Biol. Rhythms.* 11: 68-74.
- Feng, M.G., T.J. Poprawski, and G.G. Khachatourians. 1994. Production, formulation, and application of the entomopathogenic fungus *Beauveria bassiana* for insect control: current status. *Biocon. Sci. Tech.* 4: 3-34.
- Frank, J.H. and J.P. Parkman. 1999. Integrated pest management of pest mole crickets with emphasis on the southeastern USA. *Int. Pest Manag. Rev.* 4: 39-52.
- Harris, J.P. 1999. Fall mole cricket control on a golf course in south Mississippi with BotaniGard. *Arthropod Manag. Tests.* 24: 342.
- Hertl, P.T. and R.L. Brandenburg. 1998. Evaluation of *Beauveria bassiana* spore applications for control of mole cricket nymphs in turf. *Arthropod Manag. Tests.* 23: 323.

- Jaronski, S.T. and M.S. Goettel. 1997. Development of *Beauveria bassiana* for control of grasshoppers and locusts. Mem. Entomol. Soc. Can. 171: 225-237.
- Matheny, E.L., Jr. 1981. Contrasting feeding habits of pest mole cricket species. J. Econ. Entomol. 74: 444-445.
- Racke, K.D. 2000. Pesticides for turfgrass pest management: uses and environmental issues, pp. 45-64. In J.M. Clark and M.P. Kenna (eds.), Fate and Management of Turfgrass Chemicals. American Chemical Society, Washington, DC.
- SAS Institute. 2003. User's Guide, Version 9.1. SAS Institute, Cary, NC.
- Sharma, V.K. 2003. A simple computer-aided device for monitoring activity of small mammals and insects. Biol. Rhythm Res. 34(1): 3-12.
- Sharma, V.K., S.R. Lone, and A. Goel. 2004. Clocks for sex: loss of circadian rhythms in ants after mating? Naturwissenschaften. 91: 334-337.
- Sheeba, V., M.K. Chandrashekar, A. Joshi, and V. K. Sharma. 2001. A case for multiple oscillators controlling different circadian rhythms in *Drosophila melanogaster*. J. Insect Physiol. 47: 1217-1225.
- Sheeba, V., M.K. Chandrashekar, A. Joshi, and V. K. Sharma. 2002. Developmental plasticity of the locomotor activity rhythm of *Drosophila melanogaster*. J. Insect Physiol. 48: 25-32.
- Short, D.E. and P.G. Koehler. 1979. A sampling technique for mole crickets and other pests in turfgrass and pasture. Fla. Entomol. 62: 282-283.
- Taylor, T.R. 1979. Crop contents of two species of mole crickets, *Scapteriscus acletus* and *S. vicinus* (Orthoptera: Gryllotalpidae). Fla. Entomol. 62: 278-279.

- Thompson, S.R. and R.L. Brandenburg. 2004. A modified pool design for collecting adult mole crickets (Orthoptera: Gryllotalpidae). Fla. Entomol. 87(4): 582-584.
- Thompson, S.R. and R.L. Brandenburg. 2005. Tunneling responses of mole crickets (Orthoptera: Gryllotalpidae) to the entomopathogenic fungus, *Beauveria bassiana*. Env. Entomol. 34(1): 140-147.
- Thompson, S.R., R.L. Brandenburg, and J.J. Arends. 2005. Conidial viability and pathogenicity of *Beauveria bassiana* (Balsamo) Vuillemin for mole cricket (Orthoptera: Gryllotalpidae) control in turfgrass. Int. Turfgrass Soc. Res. J. 10: 784-792.
- Thompson, S.R., R.L. Brandenburg, G.T. Roberson, and B.M. Lineberger. 2006. A technique to monitor insect behavior utilizing infrared emitters and detectors. Fla. Ent. In prep.
- Ulagaraj, S.M. 1975. Food habits of mole crickets (Orthoptera: Gryllotalpidae: *Scapteriscus*). J. Ga. Entomol. Soc. 10: 229-231.
- Ulagaraj, S.M. 1976. Sound production in mole crickets (Orthoptera: Gryllotalpidae: *Scapteriscus*). Ann. Entomol. Soc. Am. 69: 299-306.
- Ulagaraj, S.M. and T.J. Walker. 1973. Phonotaxis of crickets in flight: attraction of male and female crickets to male calling songs. Science. 182: 1278-1279.
- Villani, M.G., L.L. Allee, L. Preston-Wilsey, N. Consolie, Y. Xia, and R.L. Brandenburg. 2002. Use of radiography and tunnel castings for observing mole cricket (Orthoptera: Gryllotalpidae) behavior in soil. Am. Entomol. 48: 42-50.

- Walker, T.J. and D. Ngo. 1982. Mole crickets and pasture grasses: damage by *Scapteriscus vicinus*, but not by *S. acletus* (Orthoptera: Gryllotalpidae). Fla. Entomol. 65: 105-110.
- Xia, Y., P.T. Hertl, and R.L. Brandenburg. 2000. Surface and subsurface application of *Beauveria bassiana* for controlling mole crickets (Orthoptera: Gryllotalpidae) in golf courses. J. Agric. Urban Entomol. 17: 177-189.

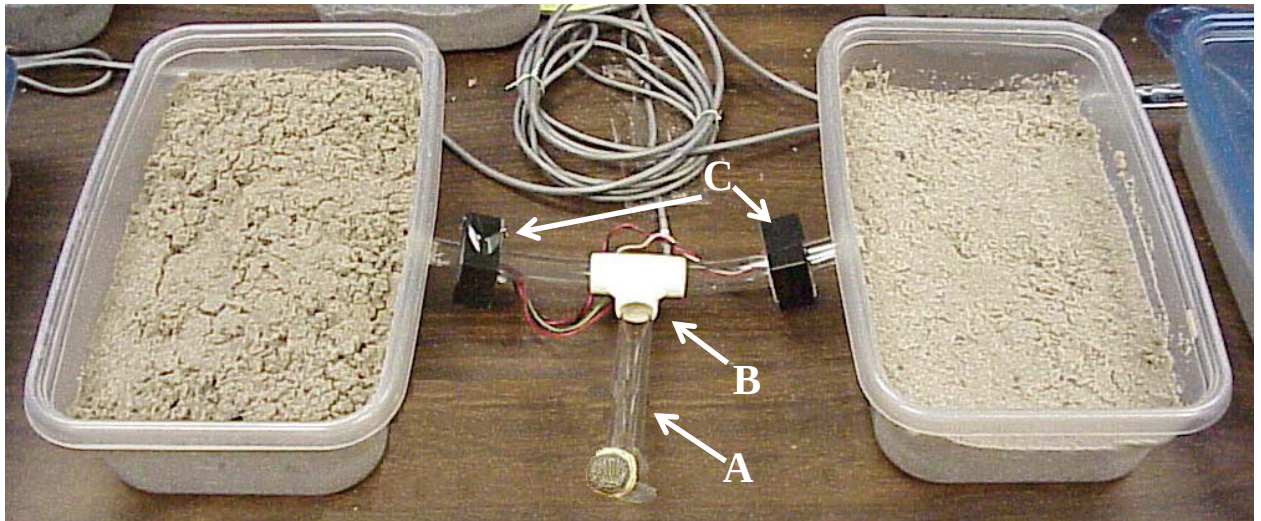


Fig. 1. Sample behavioral test unit, showing entrance tube (A), “T” connector (B), and photosensors (C). Note the extensive tunneling in the left (untreated) container in comparison to the right (treated) container

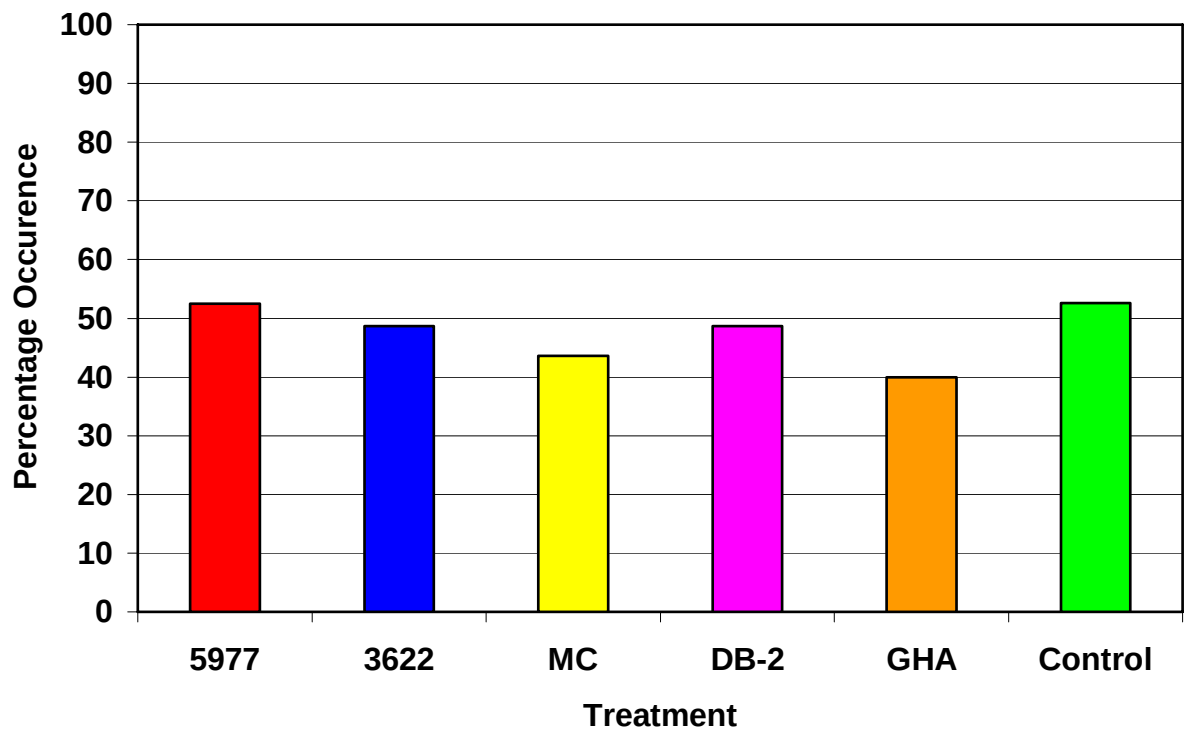


Fig. 2. Percentage occurrence of mole cricket triggering *B. bassiana*-treatment photosensor first

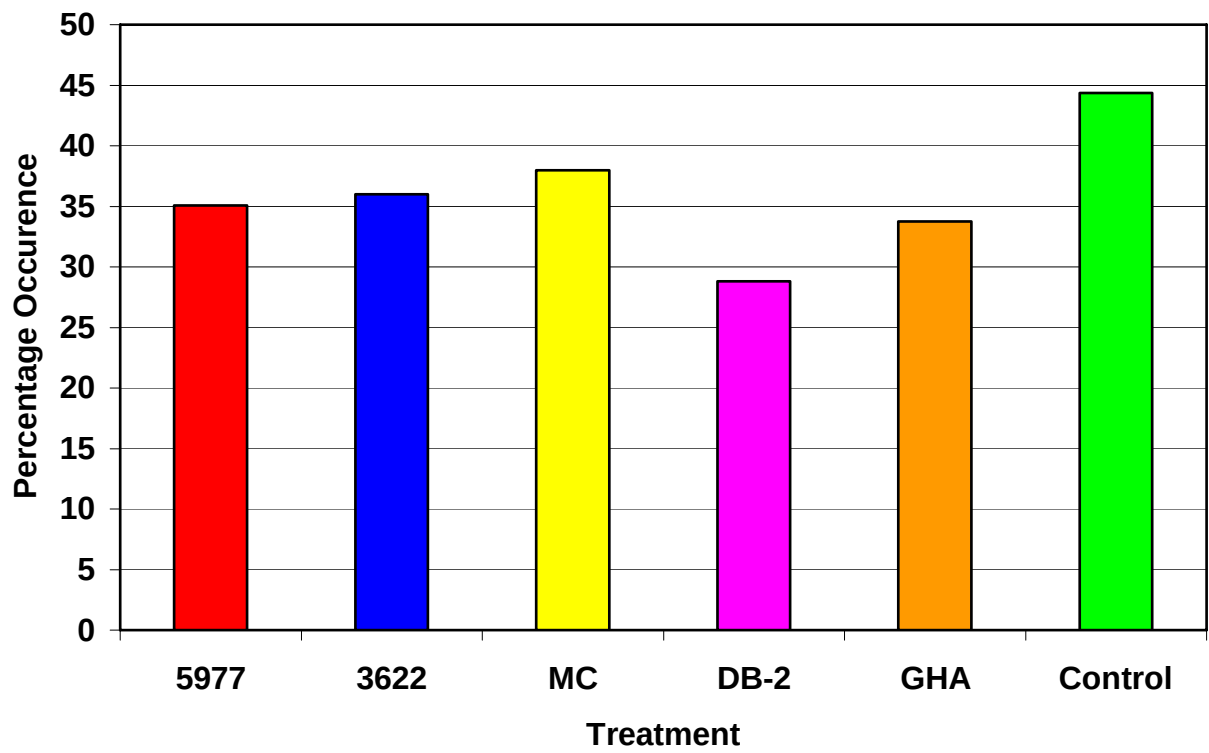


Fig. 3. Percentage of total photosensor triggers that occurred at *B. bassiana*-treated container

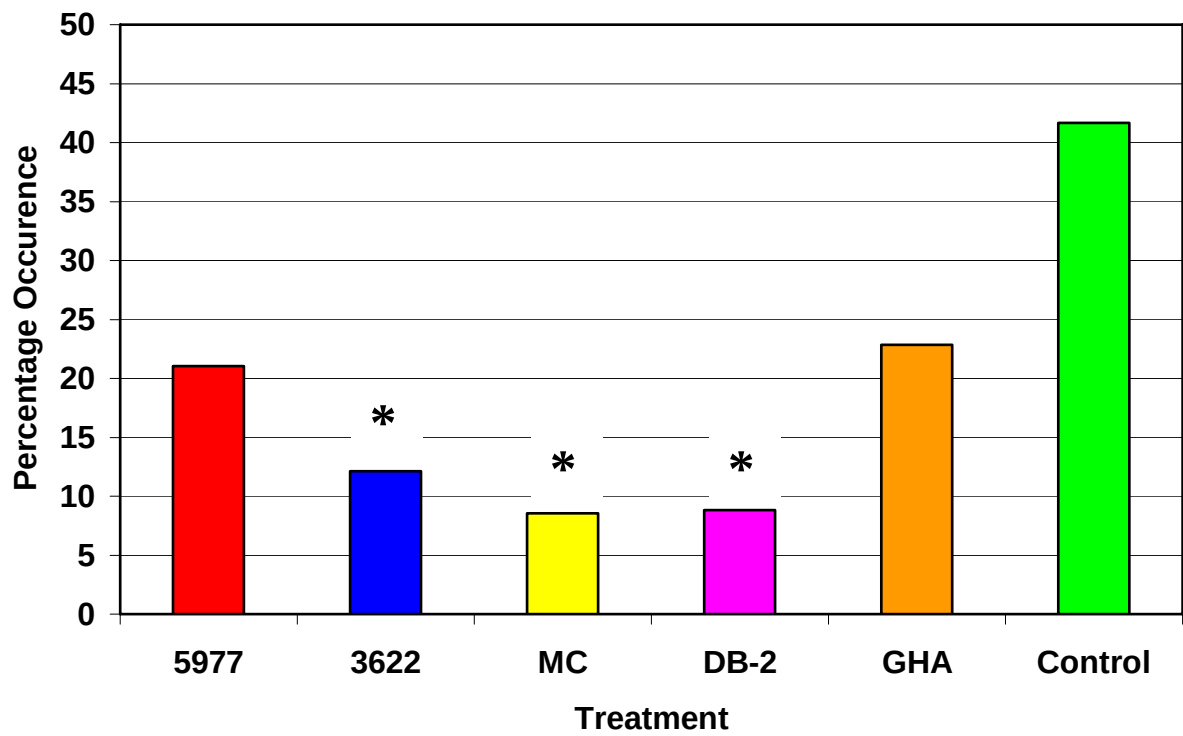


Fig. 4. Percentage occurrence of tunneling in *B. bassiana*-treated container; * indicates treatment differs significantly from control-control treatment (Likelihood Ratio χ^2 Pairwise Comparison Test, $P \leq 0.05$, SAS Institute, 2003)

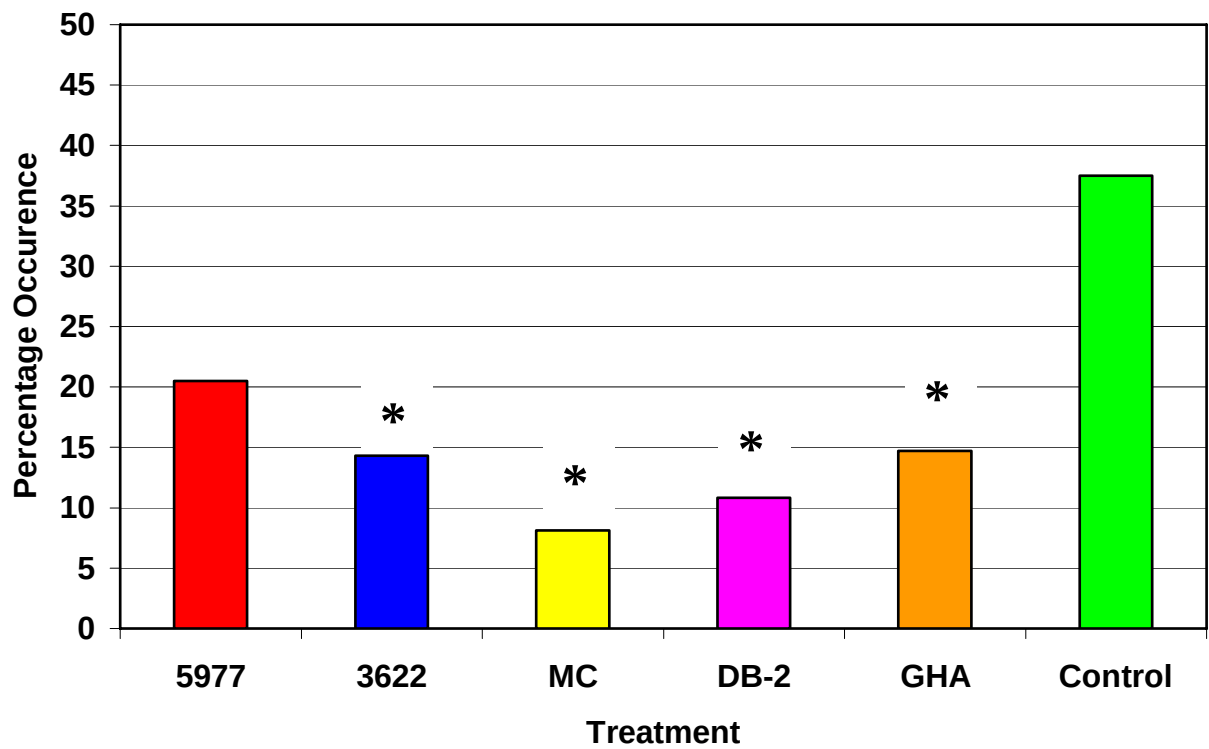


Fig. 5. Percentage occurrence of mole cricket choosing to stay in *B. bassiana*-treated container; * indicates *B. bassiana* treatment differs significantly from control-control treatment (Likelihood Ratio χ^2 Pairwise Comparison Test, $P \leq 0.05$, SAS Institute, 2003)

CONCLUSIONS

As the amount of managed turfgrass continues to grow in this country, so do the concerns over the usage of synthetic pesticides for damage control. Many of the most significant insect pests in turfgrass inhabit the soil, making high doses and repeated applications of insecticides necessary in order to reach them. This is the scenario with mole crickets, which are considered to be one of the most significant pests in turfgrass in the southeastern U.S. Since the value of turfgrass is often measured solely on its cosmetic appearance, the necessity of conventional pesticides is often questioned. A number of environmentally-safe options for mole cricket control exist, including the use of entomopathogenic fungi like the naturally-occurring soil fungus, *Beauveria bassiana*. There are a number of key factors that determine the success of this agent including contact between the cricket and the infective conidia, appropriate selection of pathogenic strains and rates, and maintenance of conidial viability in the field.

Our previous research indicates that *B. bassiana* strains isolated from orthopteran hosts are the most virulent towards mole crickets, that conidia can survive in the field for up to one month after application, and that mole crickets alter their behaviors in order to avoid contact with the conidia. In order to increase the efficacy of this fungus for mole cricket control, the research described in the preceding pages focused on these objectives: a) maximizing conidial viability in the field b) lowering application rates by making combination treatments with other insecticides and c) elucidating the mechanism behind the avoidance behaviors in order to better understand and minimize them.

A field study to measure the impact of moisture and UV exposure on conidial viability was conducted over two years in bermudagrass plots. Evaluation of viability was

achieved with the use of a two-component nucleic acid stain in conjunction with fluorescence microscopy. Results indicate that there were no differences between the two orthopteran strains evaluated, and both were able to survive for up to three weeks in the field. Comparisons of both strains under two different irrigation regimes suggest that a higher level of applied irrigation will help to maintain conidial viability over time. We hypothesize that the irrigation acted by moving the conidia further into the thatch layer and soil profile, thereby providing protection by minimizing exposure to UV light and other detrimental factors. One of the strains was formulated in four different carriers that were hypothesized to provide protection from UV degradation by either blocking or absorbing UV light (or both). An optical brightener and magnesium silicate clay added to an emulsifiable oil carrier maintained conidial viability significantly better than the clay or oil alone. This study suggests carrier formulations and management practices that can be utilized to help prolong conidial viability in turfgrass settings.

A laboratory topical bioassay was conducted with three different *B. bassiana* strains applied with diatomaceous earth (DE) or imidacloprid to test for synergistic interactions. Since the costs associated with making repeated applications of *B. bassiana* are often prohibitive and there are concerns over the use of synthetic insecticides, this study was conducted to identify combination treatments that are pathogenic but reduce rates of both products. Pathogenicity of treatments was evaluated by measuring mole cricket mortality over 28 days after treatment. Data revealed that all three strains used in combination with DE resulted in greater than additive percentage mortality and this interaction was synergistic for two of the strains. Since DE abrades the insect cuticle and absorbs cuticular lipids, it may act by aiding the entry of germinating conidia into the mole cricket hemocoel and make

nutrients more readily available for the fungus. In contrast, there was no synergy exhibited between any of the *B. bassiana* strains and imidacloprid. In fact, the combination treatments involving this insecticide resulted in less than additive effects for two of the strains, and the interaction was antagonistic for the third strain. Since DE is a relatively inexpensive product, results from this study suggest a reduced pesticide approach using DE and *B. bassiana* that could be used to increase efficacy while lowering costs.

Strain-specific mole cricket avoidance behaviors towards *B. bassiana* have been documented in the past and are hypothesized to minimize contact with conidia until they are no longer infective. Our goal in these tests was to elucidate the mechanism of detection so that strategies to eliminate or minimize these behaviors can be developed. The use of infrared emitters and detectors to monitor mole cricket movement in proximity to *B. bassiana* treatments offered a low-cost, non-invasive method for determining if contact with the conidia is necessary for detection. Results indicate the mole crickets do not require physical contact with conidia in order to detect their presence, but do need to be relatively close (less than 8 cm) in order to sense them. We also observed strain-specific behaviors that verify that some strains are more repellent to mole crickets than others. We documented a difference in behaviors between southern and tawny mole crickets, with the southern crickets exhibiting much more extreme avoidance strategies. Given the biology of these two species, these results are logical. The outcome of these studies suggests a chemosensory mechanism of entomopathogenic fungal detection and avoidance. In conclusion, the research presented here offers suggestions for ways to increase the efficacy of *B. bassiana* for environmentally-safe mole cricket control in turfgrass.

APPENDICES

G

BERMUDA GRASS: *Cynodon dactylon* (L.) Pers.

EFFICACY OF HIGH AND LOW RATES OF *BEAUVERIA BASSIANA* FOR CONTROL OF MOLE CRICKET NYMPHS IN TURF, 2004

Sarah R. Thompson

Department of Entomology

Box 7613

North Carolina State University

Raleigh, NC 27695-7613

Phone: (919) 513-2209

Fax: (919) 515- 7746

E-mail: sarah_thompson@ncsu.edu

Rick L. Brandenburg

Tawny mole cricket (TMC): *Scapteriscus vicinus* Scudder

Southern mole cricket (SMC): *Scapteriscus borellii* Giglio-Tos

The efficacy of *Beauveria bassiana* spore applications for control of mole cricket nymphs in turf was investigated in a field trial. The objective of this test was to compare the efficacy of high and low rates of *B. bassiana* to bifenthrin (Talstar Flowable) and an untreated check. 20 ft x 20 ft plots were established on the bermudagrass driving range at Sandpiper Bay Golf Course in Brunswick Co., NC, an area with a history of mole cricket infestations and signs of damage present at the time of application. The test was set up as a RCB with four

replicates of each treatment. All insecticide applications were made on 26 Aug using a CO₂-powered backpack sprayer calibrated to deliver 30 gpa. *B. bassiana* treatments included the rates of 2×10^{13} spores/acre and 5×10^{12} spores/acre. Talstar was applied at the label rate of 10 oz/1000 ft². The golf course irrigation system provided approximately 0.50 inch of water to the test site immediately following treatment applications. The four-inch soil temperature on this date was 82°F and air temperature was 86°F. Soil at the test site was classified as Leon fine sand with a pH of 4.5 and 1.67% humic matter. Soapy water flush sampling at the test site indicates that the driving range population was 84% tawny mole crickets and 16% southern mole crickets. Plots were evaluated for fresh mole cricket surface damage on 31 Aug, 9, 16, and 23 Sep using the damage grid evaluation method of Cobb and Mack (1989). A one m² grid divided into nine subsections was randomly placed in each plot and a damage rating was assigned based on the occurrence of surface damage in each of the nine subsections (with 0 = no damage and 9 = severe damage). Five ratings were made for each plot on each of the evaluation dates. The actual damage means for the five ratings are reported in the Table. All data were transformed $\sqrt{(x + 0.5)}$ prior to ANOVA and means separation by LSD t-test.

Mole cricket infestation levels at this site were very high prior to treatment applications. There were no differences between any of the pre-treatment damage rating means. On 31 Aug, there were no significant differences between any of the treatments and the untreated check. There were also no differences between any of the treatments and the untreated check on the other evaluation dates of 9, 16, and 23 Sep; however, the high rate of *B. bassiana* had numerically lower damage rating means compared to the low rate on three of

the evaluation dates. Both rates of *B. bassiana* provided the same level of control as the turfgrass insecticide Talstar, but none of the treatments lowered mole cricket activity to an acceptable level. Mole cricket nymphs are typically large and very active during the time of the year when this test was conducted, and earlier applications of any of these products may be more appropriate to achieve satisfactory levels of control.

Table 1. Mean mole cricket damage ratings

Treatment/ formulation	Rate amt/area	Mole cricket damage rating ^a			
		31 Aug	9 Sep	16 Sep	23 Sep
<i>Beauveria bassiana</i> 5 x 10 ¹²		3.30a	3.70a	4.70a	4.25a
EL	spores/acre				
<i>Beauveria bassiana</i> 2 x 10 ¹³		2.65a	3.95a	4.45a	3.40a
EL	spores/acre				
Talstar Flowable	10 oz/1000 ft ²	3.30a	3.65a	3.65a	3.25a
Untreated check	-----	3.45a	4.95a	5.70a	5.15a

Means within the same column followed by the same letter are not significantly different (LSD t-test, $P = 0.05$)

^aDamage ratings (0 = no damage, 9 = severe damage)

Table 2. Materials Tested for Arthropod Management

Common Name	Trade Name/cultivar	Concentration/ formulation	Chemical Name	Sources
Bifenthrin	Talstar Flowable	F	(2-methyl[1,1-biphenyl]-3-yl)-methyl-3-(2-chloro-3,3,3-trifluoro-1-propenyl)-2,2-dimethyl cyclopropanecarboxylate	FMC Corporation 1735 Market Street Philadelphia, PA 19103
Fungi	Strain 3622	EL	<i>Beauveria bassiana</i>	USDA-ARS Collection of Entomopathogenic Fungal Cultures Plant Protection Research Unit Tower Rd Ithaca, NY 14853 and JABB of the Carolinas, Inc. 456 East Main St. Pine Level, NC 27568-0310

G

BERMUDA GRASS: *Cynodon dactylon* (L.) Pers.

EFFICACY OF SINGLE AND MULTIPLE APPLICATIONS OF *BEAUVERIA* *BASSIANA* FOR CONTROL OF MOLE CRICKET NYMPHS IN TURF, 2005

Sarah R. Thompson

Department of Entomology

Box 7613

North Carolina State University

Raleigh, NC 27695-7613

Phone: (919) 513-2209

Fax: (919) 515- 7746

E-mail: sarah_thompson@ncsu.edu

Rick L. Brandenburg

Tawny mole cricket (TMC): *Scapteriscus vicinus* Scudder

Southern mole cricket (SMC): *Scapteriscus borellii* Giglio-Tos

The efficacy of *Beauveria bassiana* spore applications for control of mole cricket nymphs in turf was investigated in a field trial. The objective of this test was to compare the efficacies of one high rate application to multiple low rate applications of *B. bassiana*. The checks included bifenthrin (Talstar Flowable) and an untreated check. 20 ft x 20 ft plots were established on old fairway at Sea Trails Plantation in Brunswick Co., NC, an area with a history of mole cricket infestations and signs of damage present at the time of application.

The test was set up as a RCB with four replicates of each treatment. The initial treatment applications were made on 6 Jul using a CO₂-powered backpack sprayer calibrated to deliver 30 gpa. On this date, the high rate of *B. bassiana*, Talstar, and the first application of the repeated low rates of *B. bassiana* were made. The high rate of *B. bassiana* delivered 2×10^{13} spores/acre, while each of the four applications at the low rate delivered 5×10^{12} spores/acre, for a cumulative number of spores/acre equal to the high rate. The subsequent applications of the low rate of *B. bassiana* were made on 21 Jul, 1, and 17 Aug. Talstar was applied at the label rate of 10 oz/1000 ft². The golf course irrigation system provided approximately 0.50 inch of water to the test site immediately following all treatment applications. The air temperatures were 86°F on 6 Jul, 82°F on 21 Jul, 76°F on 1 Aug, and 85°F on 17 Aug. Soil at the test site was classified as Foreston fine sand with a pH of 3.9 and 0.86% humic matter. Soapy water flush sampling at the test site indicates that the fairway population was 93% tawny mole crickets and 7% southern mole crickets. Plots were evaluated for fresh mole cricket surface damage on 11, 17, 23, 31 Aug, and 7 Sep using the damage grid evaluation method of Cobb and Mack (1989). A one m² grid divided into nine subsections was randomly placed in each plot and a damage rating was assigned based on the occurrence of surface damage in each of the nine subsections (with 0 = no damage and 9 = severe damage). Five ratings were made for each plot on each of the evaluation dates. The actual damage means for the five ratings are reported in the Table. All data were transformed $\sqrt{(x + 0.5)}$ prior to ANOVA and means separation by LSD t-test.

Mole cricket infestation levels at this site appeared to be low prior to treatment applications, but this was most likely due to the inability of accurately rating the plots due to high

mowing heights. There were no differences between any of the pre-treatment damage rating means. On 11 Aug, there were no significant differences between any of the treatments and the untreated check. There were also no differences between any of the treatments and the untreated check on 17 and 23 Aug; however, all treatments had numerically lower mean damage ratings compared to the untreated check. On 31 Aug, only Talstar treated plots showed significantly less damage than the control, but this treatment did not differ from the high rate or multiple applications of the low rate of *B. bassiana*. There were no differences between any of the treatments and the untreated check again on 7 Sep. Mole cricket nymphs are typically large and very active during the time of the year when this test was conducted, and earlier applications of any of these products may be more appropriate to achieve satisfactory levels of control. Additionally, the goal of the multiple applications of *B. bassiana* was to build up field levels of conidia, but treatments may need to be applied more frequently than every two weeks to accomplish this.

Table 1. Mean mole cricket damage ratings

Treatment/ formulation	Rate amt/area	Application date(s)	Mole cricket damage rating ^a				
			11 Aug	17 Aug	23 Aug	31 Aug	7 Sep
<i>Beauveria bassiana</i> EL	5 x 10 ¹² spores/acre	6, 21 Jul, 1, 17 Aug	0.40a	2.15a	5.10a	4.05ab	7.25a
<i>Beauveria bassiana</i> EL	2 x 10 ¹³ spores/acre	6 Jul	1.00a	1.85a	4.80a	3.90ab	6.75a
Talstar Flowable	10 oz/1000 ft ²	6 Jul	0.20a	1.65a	5.25a	3.10a	6.30a
Untreated check	-----	-----	0.95a	2.75a	6.35a	5.80b	6.95a

Means within the same column followed by the same letter are not significantly different (LSD, P = 0.05)

^aDamage ratings (0 = no damage, 9 = severe damage)

Table 2. Materials Tested for Arthropod Management

Common Name	Trade Name/cultivar	Concentration/formulation	Chemical Name	Sources
Bifenthrin	Talstar Flowable	F	(2-methyl[1,1-biphenyl]-3-yl)-methyl-3-(2-chloro-3,3,3-trifluoro-1-propenyl)-2,2-dimethyl cyclopropanecarboxylate	FMC Corporation 1735 Market Street Philadelphia, PA 19103
Fungi	Strain 3622	EL	<i>Beauveria bassiana</i>	USDA-ARS Collection of Entomopathogenic Fungal Cultures Plant Protection Research Unit Tower Rd Ithaca, NY 14853 and JABB of the Carolinas, Inc. 456 East Main St. Pine Level, NC 27568-0310