Lethality of the Entomogenous Fungus *Beauveria bassiana* Strain NI8 on *Lygus lineolaris* (Hemiptera: Miridae) and its Possible Impact on Beneficial Arthropods[1]

Maribel Portilla[2], Randall Luttrell, Gordon Snodgrass, Yu Cheng Zhu, and Eric Riddick[3]

USDA Agricultural Research Service, Southern Insect Management Research Unit, 141 Experiment Station Road, P.O. Box 346, Stoneville, Mississippi 38776 USA

J. Entomol. Sci. 52(4): 352–369 (October 2017)

Abstract Bioassays were conducted to examine the pathogenicity of the fungus *Beauveria bassiana* (Balsamo) Vuillemin (Ascomycota: Hypocreales), strain NI8, against *Lygus lineolaris* (Palisot de Beauvois) and its impact on beneficial arthropods including *Apis mellifera* L., *Crysoperla rufrilabris* Burmeister, *Orius insidiosus* Say, *Hippodamia convergens* Guérin-Méneville, *Harmonia axyridis* (Pallas), *Coleomegilla maculata* De Geer, and field-collected Aranea spiders Salticidae and Thomisidae. Insects were treated with four concentrations of NI8 (3.9×10^4, 2.3×10^5, 4.2×10^6, and 1.5×10^7 spores/ml) directly via topical spray. Median lethal concentration (LC_{50}), sporulation response, and resistance ratio were estimated for all species except for the two groups of spiders. No significant differences in mortality (10 d after application) were observed among *L. lineolaris*, *A. mellifera*, and *C. rufilabris*, and all three species were highly affected when exposed to the highest concentrations of *B. bassiana* with 99.0, 98.2, and 90.0% mortality, respectively. Between 35 and 45% of the tested populations of *O. insidiosus*, *H. convergens*, field-collected crab spiders, and *C. maculata* were killed at 1.5×10^7 spores/ml; whereas only 22 and 27%, respectively, of the field-collected jumping spiders and *H. axyridis* were killed at 10 d with the same concentration. No significant differences were found between the LC_{50}s measured for *L. lineolaris* and *C. rufilabris* 10 d after application. Results suggested that *C. rufilabris* would be highly affected by the NI8 strain of *B. bassiana* when applied for control of *L. lineolaris*. In contrast, *B. bassiana* appeared to have lower effects on the other beneficial arthropods assayed at the concentrations targeted for *L. lineolaris* control.

Key Words tarnished plant bug, honey bee, lady beetle, green lacewing, spiders

The tarnished plant bug, *Lygus lineolaris* (Palisot de Beauvois), has one of the broadest documented feeding niches of any known arthropod pest (Robbins et al. 2000). This insect is a common pest of cotton in Mississippi and across the southern United States. Nymphs and adults feed on the reproductive structures of the cotton plants resulting in direct economic damage. Losses may vary depending on *L. lineolaris* population levels, environmental conditions, or efficacy of

[1]Received 25 January 2017; accepted for publication 19 March 2017.
[2]Corresponding author (email: Maribel.Portilla@ars.usda.gov).
[3]USDA Agricultural Research Service, National Biological Control Laboratory, 59 Lee Road, Stoneville, MS 38776.

insecticides targeted to control the pest (Snodgrass 1996). *Lygus lineolaris* has a remarkable ability to develop resistance to insecticides, and some organophosphates and pyrethroid insecticides no longer provide adequate control (Hollingsworth et al. 1997, Snodgrass 1996, Snodgrass and Scott 2000, Snodgrass et al. 2009). Since the late 1990s, there has been keen interest in decreased reliance on chemical insecticides for control of this insect, including efforts to find naturally occurring microbial pathogens that can be applied for biological control efforts. Entomopathogenic fungi may have the greatest potential among microbial control agents for controlling sucking insect pests such as *L. lineolaris* because of the contact mode of action (Tanada and Kaya 1993). Additionally, *L. lineolaris* is considered to be an early season pest that colonizes fields after initiation of flowering (Musser et al. 2007, Phelps et al. 1997, Scott and Snodgrass 2000). Selective microbial biopesticides could be particularly well suited for *L. lineolaris* management on wild host plants before they move to cotton and trigger chemical applications (Leland et al. 2005).

Beauveria bassiana (Balsamo) Vuillemin (Sordariomycetes: Hypocrales) is a cosmopolitan fungal pathogen of insects found in almost all ecosystems and is one of the most commonly applied mycoinsecticides for a variety of insect control purposes including high compatibility with beneficial arthropods (Kmitowa and Bajan 1982, Leyva et al. 2011, Rossoni et al. 2014, Thungrabeab and Tongma 2007, Todovora et al. 1996). In 2002, *B. bassiana* was isolated from a sporulating *L. lineolaris* cadaver recovered in the Mississippi Delta and assigned the strain identifiers TPB3 and later NI8 (Leland and Snodgrass 2005). A number of different studies using this strain reported encouraging results because of its higher activity against *L. lineolaris*, and additional research has been initiated for use of NI8 as an alternative *L. lineolaris* control measure. The native delta strain appears to be a promising isolate for control of *L. lineolaris* in the Mississippi Delta based on comparative pathogenicity to other native strains from Mississippi, Arkansas, and California, and the commercial strain GHA (i.e., lower median lethal concentration [LC_{50}], higher mortality, and conidia production) (Leland 2005, Leland et al. 2005, Leland and Snodgrass 2005, McGuire et al. 2006, Portilla et al. 2014, Ugine 2012).

Lygus lineolaris and beneficial insects are found in marginal areas near fields, ditches, and roadsides. Snodgrass (1991) found these areas as an important source of *L. lineolaris* moving into cotton when it reaches the pinhead stage of development. Uncultivated field borders and ditches are agro-ecosystems that are also favorable environments for natural enemies and other beneficial insects such as pollinators, probably because of low levels of disturbance. *Beauveria bassiana* sprays targeting *L. lineolaris* in marginal habitats undisturbed by agricultural practices could also potentially impact populations of pollinators and natural enemies. Therefore, this research examined the effect of *B. bassiana* sprays targeted at *L. lineolaris* and on adults of some of the key groups of beneficial arthropods including the following: *Apis mellifera* L. (Hymenoptera: Apidae), *Crysoperla rufilabris* Burmeister (Neuroptera: Chrysopidae), *Orius insidiosus* Say (Hemiptera: Anthocoridae), *Hippodamia convergens* Guérin-Méneville (Coleoptera: Coccinellidae), *Harmonia axyridis* (Pallas) (Coleoptera: Coccinellidae), *Coleomegilla maculata* De Geer (Coleoptera: Coccinellidae), field-collected spider species in the families of jumping spiders (Araneae: Salticidae), and crab spiders (Aranedae: Thomisidae) that may have included a number of different spider species. *Lygus*

lineolaris was used as an insect control and reference for chosen test concentrations.

Materials and Methods

Insect colonies. Adult *L. lineolaris* assessed in this study were from a colony established in 1998 at the USDA Agricultural Research Service (ARS) Biological Control Rearing and Research Unit in Starkville, MS. (Cohen 2000, Portilla et al. 2011). This colony was moved and has been maintained at the USDA ARS Southern Insect Management Research Unit (SIMRU) in Stoneville, MS since 2010. The rearing system used was described by Portilla et al. (2011), which uses a semisolid artificial diet and allows for the mass production of even-aged individuals. Insects were held in environmental chambers with 12:12 h (L:D) photoperiod, 27°C, and 60% relative humidity (RH). Mixed sex adults (50 females:50 males) that were 1–2 d old were used for bioassays.

Adults of *C. rufilabris, O. insidious,* and *H. convergens* were purchased from a commercial supplier (Biocontrol Net Work, Brentwood, TN). Mixed sex adults of *C. rufilabris* and *O. insidious* that were 2–3 d old were shipped overnight to SIMRU. *Hippodamia convergens* were of unknown age and shipped in a similar fashion. Insects were maintained collectively in the original container obtained from the commercial supplier. Insects were held in a growth chamber at 4°C, 55% RH, and a photoperiod of 0:24 h (L:D) for about 24 h before being used for bioassays. Low temperature and darkness declined insect's activity before *B. bassiana* application and facilitated handling.

Eggs of *H. axyridis* and *C. maculata* were originally obtained from colonies maintained at the USDA ARS National Biological Control Laboratory (NBCL) in Stoneville, MS. Eggs were maintained in screened petri dishes (6 cm diameter) in environmental chambers at 27°C, 60% RH and a photoperiod of 12:12 h (L:D) until hatched. Newly-emerged larvae were placed individually into 29.7-ml SOLO cups (T125 0090 Clear, SOLO CUP Co., Highland Park, IL) with a solid diet developed for *L. lineolaris* bioassays (Portilla et al. 2014a). Frozen eggs of *Helicoverpa zea* Boddie (Lepidoptera: Noctuidae) obtained from the SIMRU rearing unit facility were offered to each larva on regular basis until they reached adulthood. *Lygus* solid diet was provided as a food source for adults of both *H. axyridis* and *C. maculata*. Mixed-sex adults that were 2–3 d old of both species were used in all bioassays.

Colonies and brood *A. mellifera* were originally supplied by local beekeepers in Mississippi and Arkansas. Hives were maintained in the Stoneville Wildlife Management Area located near Stoneville, MS. Each hive was equipped with an oil trap installed at the bottom of each hive for the control of small hive beetle, *Aethina tumida* Murray (Coleoptera: Nitidulidae), and the and varroa mite, *Varroa destructor* Anderson and Trueman (Parasitiformes: Varroidae). Brood frames from the hives were evaluated, and those with more than 50% coverage of healthy brood were transferred to an incubator at 33°C, 65% RH and photoperiod of 0:24 h (L:D). Thirty newly emerged bees were transferred to each cage and maintained at 33°C in an incubator for at least 4 d before being used for bioassays. Cages were made following details found in Zhu et al. (2015). Each cage, containing 30 workers (5 d old), was supplied with a $1 \times 1 \times 2$ cm piece of Global Patties (purchased from

Table 1. Spores quantification of *Beauveria bassiana* strain NI8 by using the Scepter TM 2.0 spore counter and Software Pro

	Spores ± (SD)		
Concentrations (Spores/g)	**Spores/ml ($n = 20$)**	**Spores/application ($n = 20$)**	**Spores/mm^2 ($n = 300$)**
1.20E+11	1.5E+7 ± 1.4E+6 a	9.5E+7 ± 8.7E+6 a	386.35 ± 149.46 a
1.20E+10	4.2E+6 ± 9.2E+5 b	2.5E+7 ± 5.5E+6 b	159.35 ± 97.56 b
1.20E+09	2.3E+5 ± 5.5E+4 c	1.4E+6 ± 3.3E+5 c	77.12 ± 59.57 c
1.20E+08	3.9E+4 ± 1.6E+4 c	2.3E+5 ± 9.8E+4 c	22.73 ± 14.58 d

Means within a column followed by a different letter were significantly different at $P \leq 0.05$ (Tukey Test)

Betterbee Inc., Greenwich, NY), and 20 ml each of sugar syrup (50%, V/V) and d-H_2O in scintillation vials (Zhu et al. 2015).

Approximately 200 jumping (Aranea: Salticidae) and crab spiders (Aranea: Thomisidae) of unknown species were collected from wild hosts in marginal areas near cotton fields in the Stoneville, MS Wildlife Management Area. Collections were made with a sweep-net during various periods of the day. Collected spiders were transferred to the laboratory, selected visually into family groups that may include a number of different species, and immediately placed individually into a 29.7-mL SOLO cup with solid diet developed for *L. lineolaris* bioassays. A high diversity of spiders was collected; however, only Salticidae and Thomisidae families had sufficient numbers for setting up the essays. One or two second-instar nymphs of *L. lineolaris* were provided as needed as prey for each spider. Solid diet was provided as a food source to maintain the spider's prey and humidity for the cup. Spiders were held in a growth chamber at 27°C, 55% RH, and a photoperiod of 12:12 h (L:D) for 4 d before the bioassay. The largest and healthiest spiders (similar sizes) were used in the study.

Production of NI8 spore powder and fungal application. NI8 was produced at the USDA ARS NBCL by using a small-scale biphasic culture system for solid-substrate fermentation as described by Portilla et al. (2016). Harvested spore powder from the strain NI8 was assessed for spore germination by using the method described by Velez et al. (1997) and for the spore quantification (viable conidia/g) by using a hemocytometer 0.1 mm deep (Reichert-Bright Line, Buffalo, NY). Then, 0.5 g of harvested spore powder that contained 1.2×10^{11}, 1.2×10^{10}, 1.2×10^{9}, and 1.2×10^{8} viable spores per g were suspended in 50 mL of 0.04% Tween-80 (Sigma-Aldrich P8074) and diluted to obtain final concentrations of 1.5×10^{7}, 4.2×10^{6}, 2.3×10^{5}, and 3.9×10^{4} spores per ml (Table 1). An aliquot of 6 ml of conidia suspension per concentration was sprayed on 5 disposable microscope cover slips (22 mm^2; Fisher Scientific, Ref. No. 12-547) equally spaced in a petri plate (15 cm diameter × 3 cm deep) (Pioneer Plastic, Ref. No. 170C). The suspension was applied using a spray tower that covered an area of 38.5 cm diameter (arena) fitted with an air-atomizing nozzle (Ref. No. 1/4J) with a fluid cap (Ref. No. 2850) and air cap 70 (Spraying System Co. FN5925-001-001A). The

atomized nozzle was connected to a shielded flow meter (Thermo Scientific Gilmont ®, Ref. No. GF-2060, Vernon Hills, IL) that provided a constant airflow of 10 liters/min with a constant pressure of 12.5 kPa cm^2. After spray applications of conidia, cover slips were removed from the spray tower, and each cover slip was placed into a separate 37-mL SOLO cup (No. T25-0090) that contained 2 ml of phosphate buffered saline (water 800 ml + NaCl 8 g + KCL 0.2 g + Na_2HPO_4 1.44 g + KH_2PO_4 0.24 g). The cups with the solutions were vortexed (Mini Vortexer VWR Scientific Products Serial No. 16022) for about 1 min to wash the spores from the cover slip into the solution. When prompted, 30 μl conidia suspension + 30 μl of phosphate buffered saline was put into a 1.5-ml microcentrifuge tube and vortexed. The number of spores suspended in solution from each vial was quantified using the Scepter ™ 2.0 Cell Counter and Software Pro (Cappione et al. 2011). The process was replicated five times per concentration and three pseudoreplicated counts were done per cover slip (300 spore counts) (4 concentrations × 5 replicate Petri dishes × 5 cover slips per petri dish × 3 replication per cover slip). For each replicated spray concentration, a suspension of 50 ml was prepared (25 vials of 50 ml suspensions). The spore quantifications were studied by analysis of variance (SAS Institute 2013). The numbers of spores delivered per mm^2 per aliquot of 6 ml of suspension per concentration are shown in Table 1.

Bioassay procedure. Stock conidia suspension was serially diluted to obtain four concentrations of 1.5×10^7, 4.2×10^6, 2.3×10^5, and 3.9×10^4 spores/ml (conidia 97% viability). Each treatment suspension and water control treatment was applied to each of four replicate groups of 30 mixed-sex adult *L. lineolaris* that were 2–3 d old (20 groups [G], 600 individuals [I]), 30 workers 10–12 d old of *A. mellifera* (20 G, 600 I), 10 2–3 d old mixed-sex adults of *H. axyridis* and *C. maculata* (20 G, 200 I per species), and 10 adults (unknown age) of *H. convergens* and *O. insidiosus* (20 G, 200 I per species). Some of the field-collected crab and jumping spider groups were not enough to test all the *B. bassiana* concentrations. Therefore, for each spider family, only 4 groups of 10 individuals were treated at the highest *B. bassiana* concentration. Each replicate group of species, except for *A. mellifera*, were held at 4°C in an environmental chamber for 2–5 min periods to minimize the insects' activity before *B. bassiana* application. Each test group of insects and spiders was randomly distributed in a petri plate (15 × 3 cm) (same size plate used to assess spore quantification and germination), which was positioned in the center of the sprayed arena of the spray tower. Treatment concentrations were similarly delivered in a 6 ml spray volume by using the protocol mentioned before for *L. lineolaris*. Control insects were sprayed with 6 ml of water. Due to the difficulties of managing honey bees outside of the container, jars containing a group of 30 workers were placed in the center of the covered area of the spray tower and sprayed through the cut lid of the jar (8.9 cm diameter mouth covered with 8-mesh metal screen). These containers were designed by Zhu et al. (2015) to facilitate spray applications. Insects and spiders were sprayed when they were available (different dates) by using the same spore powder prepared for the spore quantification.

After *A. mellifera* were sprayed with *B. bassiana,* containers were transferred to an incubator at 33°C, 65% RH, and photoperiod of 0:24h (L:D). Sugar solution and water vials were provided as described before. Due to the characteristics of social insects, bioassays were set up to mimic environmental characteristics commonly

found in a hive (e.g., behavior, temperature, photoperiod). Maintaining a group of 30 worker bees in a container replicates frequent social contact, as would be found in a hive. *Orius insidiosus* were individually placed into 37-ml SOLO cups with a 3-cm piece of fresh green bean, *Phaseolus vulgaris* L., as a food source. These beans were changed every 2 d until the end of the assay. The remaining species of both insects and the two spider groups were placed individually into 37-ml SOLO cups with solid *Lygus* spp. diet. Adults were examined daily for 10 d to assess mortality and 14 d to assess sporulation. Eggs oviposited by both *C. rufilabris* and the coccinellids were removed to avoid predation (newly emerged larvae would feed on their mothers and affect mortality). Dead insects were retained in the same cup until completion of the 10-d trial to observe sporulation. Relative humidity of approximately 80% was maintained within a diet SOLO cup (bioassay arena). This RH reportedly provides the proper conditions for mycelial growth (Portilla et al. 2016, Portilla et al. 2014a). Percentage sporulation (the number of cadavers producing NI8 spores) was calculated for the total number of dead cadavers. No fungal growth was observed on *A. mellifera* cadavers when they were inside the jar; therefore, to measure sporulation, infected cadavers were removed from inside the jar and placed individually onto a plate of Sabouraud Dextrose Agar Premix (10g/liter peptone, 40 g/liter dextrose, 15 g/liter agar, 2 g/liter yeast extract). Plates with *A. mellifera* cadavers were kept for 3–5 days in an incubator at 27°C until sporulation was observed.

Statistical analysis. All data were analyzed using SAS system software (SAS 2013). Randomized complete block designs with factorial arrangements were used as follows: (1) 5×4 (concentrations $\times$ replicates) for each group of insect species for mortality and sporulation, (2) 7×4 (insect species $\times$ replicates) for cumulative mortality observed at each concentration for all insects, and (3) 9×4 (species of insects and spider families $\times$ replicates) for the cumulative mortality at the highest concentration. Each insect species and the two spider families were compared to the mortality of *L. lineolaris* as a positive insect control and analyzed using a one-way ANOVA followed by Tukey's HSD. Nonparametric estimates of the survival function for each insect species were compared between concentrations by using PROC LIFETEST (SAS 2013). Statistical differences in the survival of each species were declared based on the log-rank statistic. A nonparametric estimate of survival function for each insect species and the two spider families treated with the highest concentration was compared using PROC LIFETEST. To calculate slops and estimate LC_{50}, sporulation response (SR_{50}), and resistance ratio (RR_{50}) of *B. bassiana* strain NI8, technical powder, mortality, and sporulation for each insect species from bioassays were corrected for control effects by using Abbott's formula (Abbott 1925). Corrected data from the bioassays were analyzed using a PROC PROBIT (SAS 2013). Bioassays were considered significant when the slope of the line was significant ($P < 0.05$). Probit trends with significant ($P = <0.05$) goodness of fit test were evaluated to ensure the form of the trend being modeled was appropriated. RR_{50} and confidence intervals were calculated using Robertson and Priestler (1992). The RR_{50} compares the LC_{50} and SR_{50} values for the beneficial insects tested to those from *L. lineolaris*, which was used as insect control. LC_{50} and SR_{50} values for each insect species were contrasted using linear regression (SAS 2013).

Results

Effect of *B. bassiana* strain NI8 on the survival of *L. lineolaris* and beneficial arthropods. *Beauveria bassiana* strain NI8 affected survival of all studied species. Fig. 1A, B, C, E, F, and G indicated significant differences among concentrations for all insects, except for *O. insidiosus* (Fig. 1D). Adults treated with higher rates died faster than those treated with lower rates. *Lygus lineolaris* and *C. rufilabris* were highly affected at all test concentrations (Fig. 1A), whereas *A. mellifera* was only affected at the highest concentration (1.5×10^7 spores / ml) (Fig. 1B, C). Lady beetles had a similar survival rate (Fig. 1E, F, G). Significant differences in survival were also observed among species treated with the highest concentration (Wilcoxon $\chi^2 = 34.52$; df = 8; $P < 0.0001$). *Lygus lineolaris* survival trend declined faster than any other species, followed by *C. rufilabris* and *A. mellifera*. However, the survival lifetest analysis indicated that *A. mellifera* had the lowest survival rate when exposed to the highest concentration 10 d after application. The least affected species were *H. convergens* and the field-collected jumping spider families that may have included a number of different species (Fig. 2).

Cumulative mortality and infection of *B. bassiana* strain NI8 on *L. lineolaris* and beneficial arthropods. Under laboratory conditions and by direct application, both *L. lineolaris* and the predator *C. rufilabris* were highly susceptible to *B. bassiana* strain NI8 at all test concentrations: 3.9×10^4 ($F = 16.53$; df = 6, 3; $P < 0.0001$), 2.3×10^5 ($F = 18.99$; df = 6, 3; $P < 0.0001$), 4.2×10^6 ($F = 54.18$, df = 6, 3; $P < 0.0001$), and 1.5×10^7 ($F = 11.46$, df = 8, 3; $P < 0.0001$). Although the earliest mortality recorded among species tested was for *L. lineolaris* and *C. rufilabris* at all concentrations including lower concentrations, no significant differences were observed with the highest concentration after 10 d among *A. mellifera, L. lineolaris*, and *C. rufilabris* (mortality percentages [±SE] were 99.00 ± 1.0, 98.30 ± 1.8, and 90.0 ± 7.1, respectively) (Table 2, Fig. 2). The other six species tested had mortality rates below 45% mortality at the 1.5×10^7 test concentration, with *H. axyridis* and jumping spiders having mortality rates of 27.5 and 22.5%, respectively. There were no significant differences among susceptibility of these test groups. All species exposed to the lowest concentration test (3.9×10^4) did not significantly differ from those exposed to the water control, except for *C. rufilabris* and *L. lineolaris* (Table 2). The regression analysis (cubic trend model) with mortality and sporulation percentages per concentration per insect species showed a significant correlation of these two dependent variables with spore concentration tested (Fig. 3). Mortality and sporulation increased when spore concentrations increased, and r^2 values ranged from 0.945 to 0.999. Sporulation on *L. lineolaris* was higher than any other insect at all concentrations. Significant differences in sporulation were found among species at all concentrations: 3.9×10^4 ($F = 8.98$; df = 6, 3; $P < 0.0001$), 2.3×10^5 ($F = 10.43$; df = 6, 3; $P < 0.0001$), 4.2×10^6 ($F = 26.09$; df = 6, 3; $P < 0.0001$), and 1.5×10^7 ($F = 6.45$; df = 8, 3; $P = 0.0002$).

Mortality and sporulation response of *L. lineolaris* and beneficial arthropods to *B. bassiana* strain NI8. The LC_{50}s and SR_{50}s for conidia, as determined by probit analysis, indicated variability among adults of the species tested (Tables 3, 4). Dose-ratios for mortality obtained for *L. lineolaris* were similar to *C. rufilabris,* but both were higher than those found for the other insects. All

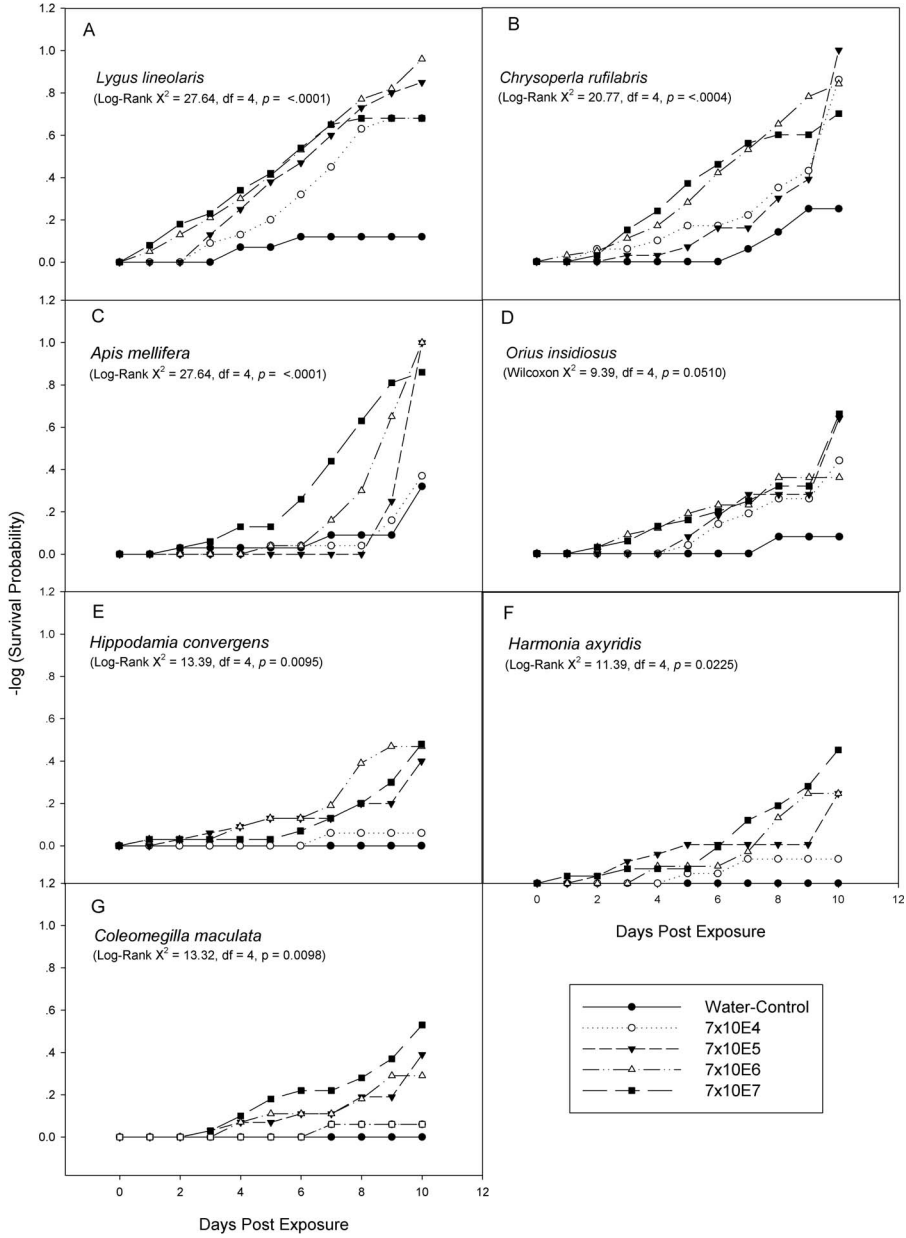

Fig. 1. Survival probability of *Lygus lineolaris* and beneficial arthropods exposed to *Beauveria bassiana* at four different concentration tests under laboratory conditions. $P = 0.05$, LIFETEST of Equality Over Strata.

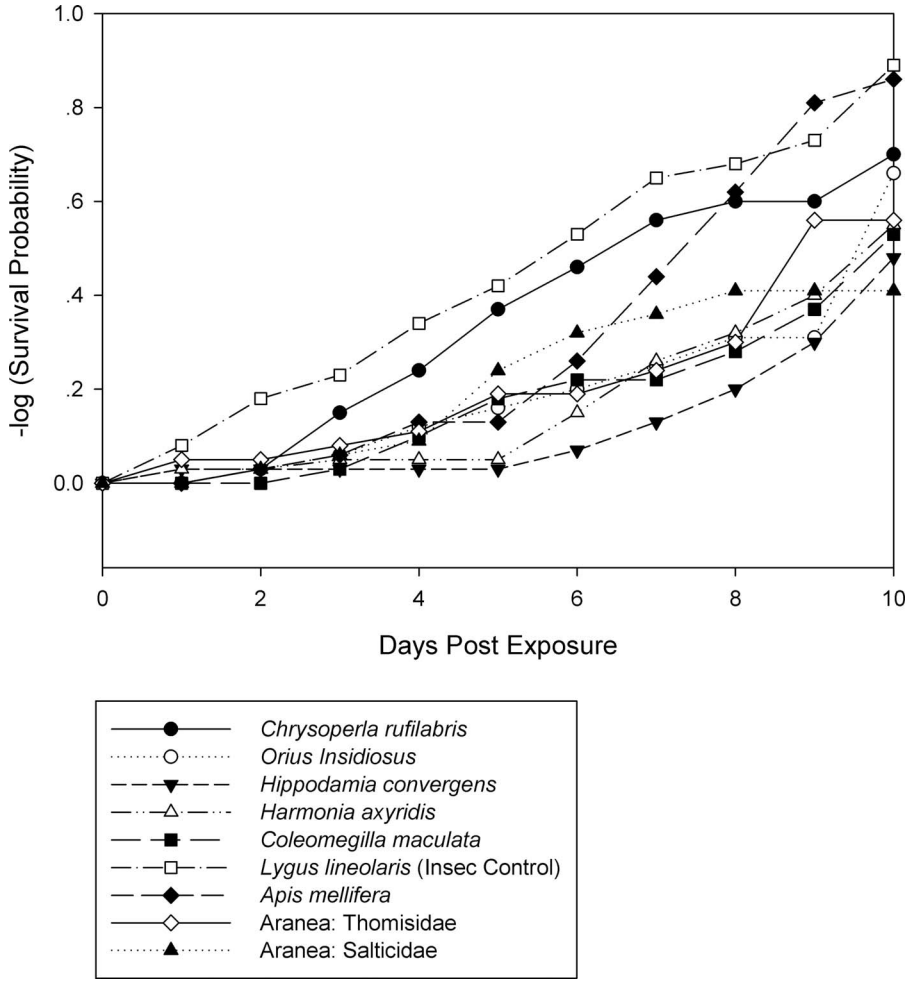

Fig. 2. Survival probability of *Lygus lineolaris* and beneficial arthropods exposed to *Beauveria bassiana* at the 1.5×10^7 concentration tested under laboratory conditions. $P = 0.05$, LIFETEST of Equality Over Strata.

beneficial arthropods treated with all *B. bassiana* concentrations except for *C. rufilabris* were tolerant of the lower concentrations tested (4.2×10^6, 2.3×10^5, 3.9×10^4) (Fig. 3). At 1.5×10^7, NI8 eventually killed 22–45% of the lady beetles, collective spider groups, and *O. insidiosus* at 10 d. Ninety to 95% of *C. rufilabris*, *L. lineolaris*, and *A. mellifera* were killed 10 d after treatments with the 1.5×10^7 concentration of *B. bassiana*. The SR_{50} for *L. lineolaris* was lower than those for other insects treated with the fungus.

Table 2. Mortality percentage of *Lygus lineolaris* and beneficial arthropods by direct spray with *Beauveria bassiana* strain NI8 at different concentrations.

	Beauveria bassiana concentrations (spores/mm^2) (means ± SE)				
Arthropod	**Water control**	**3.9×10^4**	**2.3×10^5**	**4.2×10^6**	**1.5×10^7**
Lygus lineolaris	4.17 ± 1.83 d	20.00 ± 3.67 c	50.00 ± 4.58 b	91.67 ± 2.53 a	98.33 ± 1.17 a
Chrysoperla rufilabris	10.00 ± 4.80 c	37.50 ± 7.75 b	42.50 ± 7.92 b	82.50 ± 6.08 a	90.00 ± 4.80 a
Apis mellifera	4.17 ± 1.83 c	4.17 ± 1.83 c	8.33 ± 2.53 c	27.50 ± 4.09 b	99.17 ± 0.83 a
Orius insidiosus	2.50 ± 1.50 b	15.00 ± 5.18 ba	20.00 ± 6.41 ba	27.50 ± 7.15 a	35.00 ± 7.64 a
Hippodamia convergens	5.00 ± 1.49 b	5.00 ± 1.49 b	15.00 ± 5.72 ba	30.00 ± 7.34 a	37.50 ± 7.75 a
Harmonia axyridis	0.00 ± 0.00 b	5.00 ± 1.49 ba	15.00 ± 5.72 ba	15.00 ± 3.72 ba	22.50 ± 6.69 a
Coleomegilla maculata	0.00 ± 0.00 b	2.50 ± 2.50 b	7.50 ± 3.22 ba	15.00 ± 3.72 ba	22.50 ± 6.69 a
Aranea: Thomisidae	2.50 ± 1.40 b	n/a	n/a	n/a	40.00 ± 7.80 a
Aranea: Salticidae	0.00 ± 0.0 0b	n/a	n/a	n/a	28.00 ± 7.20 a

Means ± SD followed by the same letter in each row are not significantly different ($P < 0.05$ Tukey test)
n/a = No application was done

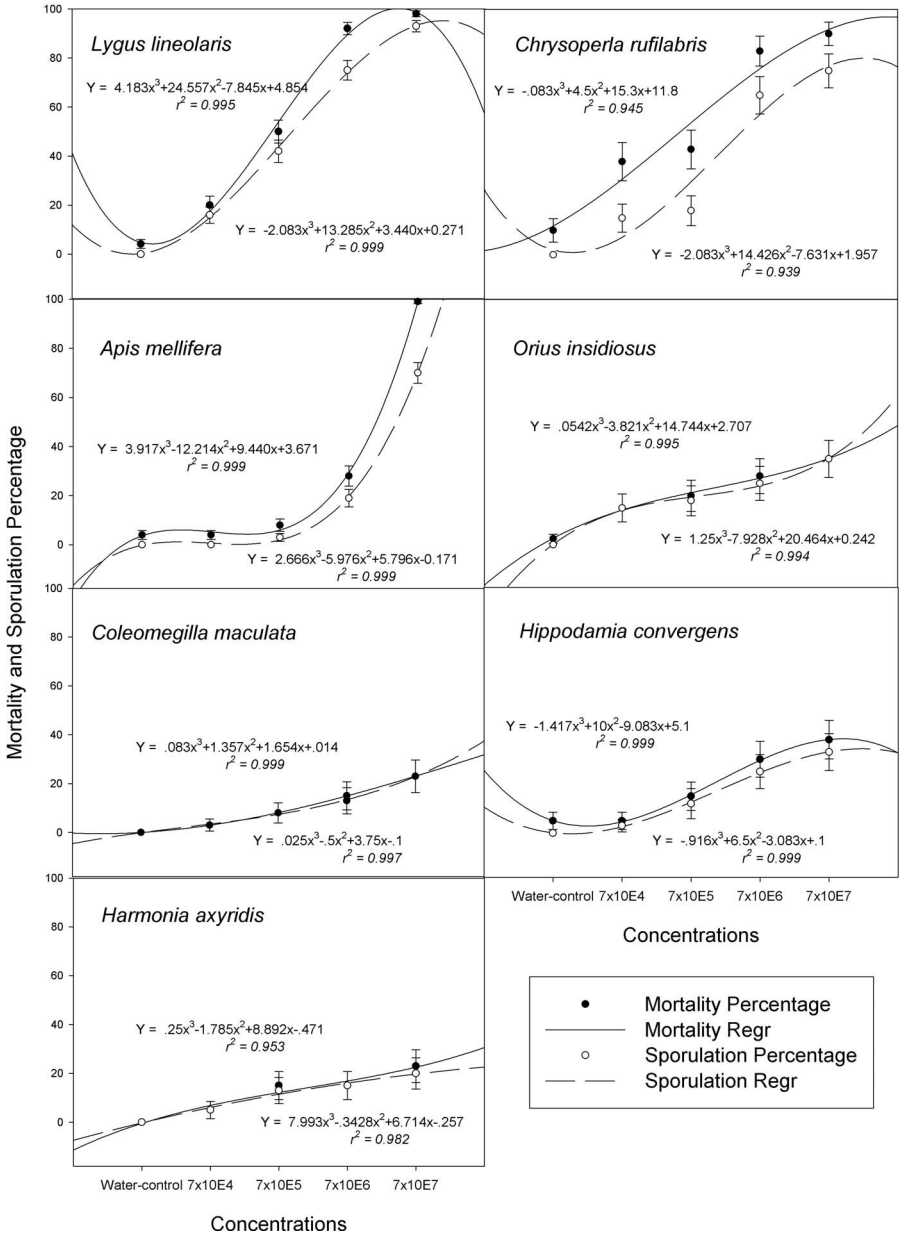

Figure 3. Regression (GLM) (Cubic Trend Model) analysis predicting probability percentage of *Beauveria bassiana* sporulation and mortality of *L. lineolaris* and beneficial arthropods as a function of the dose level.

Table 3. Mortality response (LC_{50}) of *Lygus lineolaris* and beneficial arthropods treated with *Beauveria. bassiana* strain NI8 applied at four concentrations. Concentration response (conidia/mm^2)

				Probit Trend				
				Test for Slope[2]		Test for GoF[3]		
Species	*n*	Slope ± SE	LC_{50} (95% CI)[1]	χ^2	$P > \chi^2$	χ^2	$P > \chi^2$	RR_{50} (95% CI)[4]
Lygus lineolaris (insect control)	600	1.06 ± 0.13	57.23 (43.86–71.27)	72.93	<0.0001	2.01	0.0137	1
Chrysoperla rufilabris	200	0.71 ± 0.15	72.74 (37.32–108.86)	22.01	<0.0001	1.14	0.3126	1.04 (0.58–1.87)
Apis mellifera	600	3.47 ± 0.46	196.24 (181.85–214.12)	57.75	<0.0001	1.12	0.3265	3.44 (2.72–4.34)
Hippodamia convergens	200	0.52 ± 0.17	758. 51 (368.35–10,293)	9.29	0.0023	0.59	0.8721	12.22 (4.31–34.61)
Orius i nsidiosus	200	0.03 ± 0.02	2,134 (464.78–3.81E18)	4.47	0.0346	0.82	0.6462	33.69 (2.28–496.69)
Coleomegilla maculata	200	0.37 ± 0.16	2,291 (619.15—547,730,835)	5.33	0.0211	0.57	0.8867	40.99 (4.62–363.49)
Harmonia axyridis	200	0.2 5± 0.13	5,037 (-)	3.64	0.0565	0.57	0.8913	90.14 (2.07–3,930.89)

[1] LC_{50} values are in conidia/mm^2; mortality was scored at 10 d.
[2] Test for slope significance indicates dose affects mortality or sporulation or both.
[3] Test for Goodness of Fit (GoF) significance indicates error from Probit trend is greater than expected for simple binomial response.
(4) RR_{50} (Resistance ratio) and 95% CI calculated using formula from Robertson and Priesler (1992). Differences amongst LC_{50} values are significant if 95% CI do not include 1.0. RR_{50} compare the LC_{50} for beneficial arthropods to the LC_{50} for *L. lineolaris* as a control insect.
(-) Values for CI not calculated.

Table 4. Sporulation response (SR_{50}) on *Lygus lineolaris* and beneficial arthropods treated with *Beauveria bassiana* strain NI8 applied at four concentrations

Concentration response (conidia/mm^2)								
				Probit Trend				
				Test for Slope[2]		Test for GoF[3]		
Species	*n*	Slope ± SE	SR_{50} (95% CI)[1]	χ^2	$P > \chi^2$	χ^2	$P > \chi^2$	RR_{50} (95% CI) [4]
Lygus lineolaris (Insect Control)	600	0.81 ± 0.09	69.83 (48.25–91.67)	76.23	<0.0001	1.14	0.3133	1
Chrysoperla rufilabris	200	0.74 ± 0.19	93.58 (32.98–150.22)	15.64	<0.0001	1.17	0.2849	1.33 (0.66–2.69)
Apis mellifera	600	1.05 ± 0.15	241.25 (185.89–331.08)	49.31	<0.0001	2.81	0.0003	3.44 (2.72–4.34)
Hippodamia convergens	200	0.41 ± 0.12	636.26 (264.16–6,833)	10.88	0.001	1.02	0.4288	3.45 (2.30–5.18)
Orius insidiosus	200	0.23 ± 0.15	2,363 (-)	2.52	0.1122	0.77	0.7028	33.84 (1.71–672.45)
Coleomegilla maculata	200	0.42 ± 0.29	2,384 (-)	2.07	0.1502	0.51	0.9343	34.13 (2.37–492.31)
Harmonia axyridis	200	0.24 ± 0.13	8,179 (-)	3.64	0.0565	0.79	0.6791	117.12 (1.23—11,087.13)

[1] SR_{50} values are in conidia/mm^2; sporulation was scored at 14 d.
[2] Test for slope significance indicates dose affects sporulation.
[3] Test for Goodness of Fit (GoF) significance indicates error from Probit trend is greater than expected for simple binomial response.
[4] RR_{50} (Resistance ratio) and 95% CI calculated using formula from Robertson and Priesler 1992). Differences amongst SR_{50} values. are significant if 95% CI do not include 1.0. RR_{50} compare the SR_{50} for beneficial arthropods to the SR_{50} for *L. lineolaris* as a control insect.
(-) Values for CI not calculated.

Discussion

Results indicated that pathogenicity and virulence of NI8 varied depending on the species targeted by the spray. In this study, *B. bassiana* strain NI8 was found to be pathogenic to both *L. lineolaris* and beneficial arthropods. However, it was also shown that *B. bassiana* selectivity was evident at some concentrations and that relatively low virulence was observed against *O. insidiosus*, *C. maculata*, *H. axyridis*, *H. convergens*, and the field-collected spiders visually classified Salticidae and Thomisidae, as compared to virulence in *L. lineolaris*. These results were supported by Thungrabeab and Tonga (2007), Broza et al. (2001), and Dromph and Vestergaard (2002) who noted that *B. bassiana* was non-pathogenic to *Coccinella septempunctata* L. (Coleoptera: Coccinellidae), *Chrysoperla carnea* Stephens (Neuroptera: Chrysopidae), and *Dicyphus tamaninii* Wagner (Hemiptera: Miridae) as well as a beneficial soil insect, *Heteromurus nitidus* Templeton (Collembola: Entomobryidae). James and Lighthart (1994) found that *B. bassiana* may have the potential to infect *H. convergens*, but they did not report mortality. Magalhaes et al. (1988) reported that *B. bassiana* caused mycosis in 60% of *C. maculata* adults when conidia were applied directly to the insect. Those results are comparable to our present observations where *C. maculata* was more susceptible to the highest concentration than *H. convergens*. *Harmonia axyridis* showed more tolerance to *B. bassiana* at the highest concentration where 22.5% mortality was observed. Mortality of 35% was observed for *O. insidiosus* at the 1.5×10^7 concentration, which is more than that reported from Scott and Snodgrass (2001). They reported 6.3% mortality by using direct sprays of *B. bassiana* at 1.5×10^7 spores/ml. The higher mortality obtained in the present study may have been influenced by the frequent manipulation of food source (green beans), which could also impact the survival trends (Fig. 1D) with no significant differences among concentration analyzed by PROC LIFETEST.

It is evident that *B. bassiana* NI8 will impact sprayed insects of several species differently even at the same concentration (Fig. 3). Todovora et al. (1996) suggested that different ecological host ranges of some entomopathogenic fungal isolates were evident, and that co-evolution between host and pathogens could partially explain differences in susceptibility found in many previous reports (Donegan 1989, Leyva et al. 2011, Ludwig and Oetting 2001, Mahdavi et al. 2013, Roy and Cotrell 2008). Inglis et al. (2001) indicated that selection of virulent genotypes of the pathogens is an important aspect of enhancing microbial control of insects with entomopathogenic fungi. This selective adaptation may have increased ability to sporulate on the host cadaver. However, in this study, mortality and sporulation trends were similar among *L. lineolaris* and *C. rufilabris*. Mortality of *C. rufilabris* was higher at lower concentrations than that of *L. lineolaris*, indicating that this predator will need fewer spores of *B. bassiana* to be killed than those needed for *L. lineolaris*. Interestingly, the sporulation trend for *L. lineolaris* was greater, indicating that more spores will be needed for *C. rufilabris* than *L. lineolaris* to complete the sporulation process (Fig. 3; Tables 3, 4). No significant differences were evident among the LC_{50}s measured for *L. lineolaris* (57.23 viable spores per mm^2) and *C. rufilabris* (72.74 viable spores per mm^2). A higher LC_{50} was needed for *A. mellifera* (196.24 viable spores per mm^2), and a 12.22-fold greater LC_{50} was

needed for *H. convergens*. Even greater LC_{50}s were needed for the other species, indicating some selectivity of NI8 for *L. lineolaris*.

Apis mellifera was the second most susceptible beneficial species after *C. rufilabris*. A concentration of 1.5×10^7 spores/ml killed 100% of the treated *A. mellifera* population. However, low susceptibility was evident at the lowest test concentrations including 4.2×10^6 spores/ml (Fig. 1C; Fig. 3A, B, C; Fig. 3). These results corroborated research by Al-Mazra'awi (2007) who noted that *A. mellifera* acquired doses of conidia when crawling through dispensers filled with inocula of *B. bassiana* at concentrations of 1.9×10^6, 1.3×10^6, and 3.3×10^5 with no obvious impacts on survival. Therefore, the low susceptibility to low doses could be a positive observation for NI8 use, because *A. mellifera* adults will seldom encounter spores in the field at commercial doses (2×10^{13} per 378.54 L BotaniGard ® 22WP). Similar results apply to jumping and crab spiders as well as adult lady beetles.

The test results with the field-collected and visually sorted Salticidae suggested low mortality percentage (28 ± 7.2[SE]) as compared to *L. lineolaris*. However, the field-collected and visually sorted Thomisidae had 40% mortality (Table 2). These spider groups are important, and Young et al. (1989) reported 29 families for Mississippi and 26 for Washington Co., MS. The families with the largest number of species in Washington Co. are Salticidae (47 species) and Thomisidae (11 species), supporting that our samples may have included a wide diversity of species. Crop insect pests, such *L. lineolaris*, occupying these habitats are probably exposed to considerable predation by spiders. Young et al. (1989) mentioned that management of these habitats to conserve and enhance spiders and other predators could have a significant effect on crop pest populations. Additional studies may be needed to more carefully evaluate the impact of NI8 on spider populations.

The results of this study suggest that the control of *L. lineolaris* with the entomopathogenic fungus *B. bassiana* strain NI8 will require significant ecological understanding of the interactions among the targeted host, fungal isolate, and the environment. While we must consider the economic realities of production and formulation of entomopathogenic fungi, for their use in the field, we must also be mindful of the ecological constraints and requirements for consistent insect infection and control. These experiments were conducted in the laboratory, but confirmation in the field is needed. This study indicates that the Mississippi Delta native strain NI8 may be suitable for control of *L. lineolaris*, not only because of its high pathogenicity against the targeted pest, but also for its selectivity against some beneficial arthropods.

Acknowledgments

The authors thank Tabatha Nelson, USDA ARS SIMRU for maintaining the *L. lineolaris* colony and *B. bassiana* strain NI8 spore production, and Henry Winter (SIMRU) for providing lepidopteran eggs. A special thanks to Cristofer Gutierrez (student trainee, SIMRU) for processing data and Tamiya Phillips (Summer Student, SIMRU) for the complete evaluation of the honey bee bioassay. We are also grateful to Dr. Katherine Parys and Dr. Nathan Little for their comments on an early version of this manuscript. Use of commercial and trade names or both does not imply approval or constitute endorsement by the USDA or the ARS.

References Cited

Al-mazra'awi, M.S. 2007. Impact of the entomopathogenic fungus *Beauveria bassiana* on the honey bee, *Apis mellifera* (Hymenoptera: Apidae). J. Agric. Sci. 3: 7–11.

Broza, M., P.M. Pereira and J.L. Stimac. 2001. The non-susceptibility of soil Collembola to insect pathogens and their potential as scavengers of microbial pesticides. Pedobiologia 45: 523–534.

Cappione, A., E. Crossley, N. Thirumalapura and D. Hoover. 2011. Immuno-monitoring using the Sceptertm 2.0 Cell Counter and Software Pro. Application Note-EMD Millipore. 11 pp.

Cohen, A.C. 2000. New olidic production diet for *Lygus hesperus* Knight and *L. lineolaris* (Palisot de Beaouvois). J. Entomol. Sci. 35: 301–310.

Donegan, K. 1989. Effect of several stress factors on the susceptibility of the predatory insect, *Chrysoperla carnea* (Neuroptera: Chrysopidae), to the fungal pathogen *Beauveria bassiana*. J. Invertebr. Pathol. 54: 79–84.

Dromph, M.K. and S. Vestergaard. 2002. Pathogenicity and attractiveness of entomopathogenic hyphomycetes fungi to Collembolans. Appl. Soil Ecol. 21: 197–210.

Hollingsworth, R.G., D.C. Steinkraus and N.P. Tugwell. 1997. Responses of Arkansas population of tarnished plant bug (Heteroptera: Miridae) to insecticides, and tolerance differences between nymphs and adults. J. Econ. Entomol. 1: 21–26.

Inglis, G.D., M. Goettel, M. Butt and H. Strasser 2001. Use of hyphomycetous fungi for managing insect pests, Pp. 23–69. *In* Butt, Jackson and Magan (eds). Fungi as biocontrol agents: progress, problems and potential. CABI Publishing, Oxforshire, UK.

James, R.R. and B. Lighthart. 1994. Susceptibility of the convergent lady beetle (Col., Coccinellidae) to four entomopathogenic fungi. Environ. Entomol. 23: 190–192.

Kmitowa, K. and C. Bajan. 1982. Pathogenicity level of various strains of *Beauveria bassiana* (Bals.) Vuill. Pol. Ecol. Stud. 8: 409–417.

Leland, J.E. 2005. Characteristics of *Beauveria bassiana* isolates from *Lygus lineolaris* population of Mississippi. J. Agri. Urban Entomol. 22: 57–72.

Leland, J.E. and G.L. Snodgrass. 2005. Prevalence of naturally occurring *Beauveria bassiana* in *Lygus lineolaris* (Heteroptera: Miridae) populations from wild host plants of Mississippi. J. Agric. Urban Entomol. 21: 157–163.

Leland, J.E., M.R. McGuire, J.A. Grace, S.T. Jaronski, M. Ulloa, Y.H. Park and R.D. Plattner. 2005. Strain selection of a fungal entomopathogen, *Beauveria bassiana*, for control of plant bugs (*Lygus* spp.) (Heteroptera: Miridae). Biol. Control. 35: 104–114.

Leyva, O.E., E.M. Villalon, R.A. Avila and D.B. Bulet. 2011. Susceptibilidad de *Chrysopa exterior* Navas a *Beauveria bassiana* (Blasamo) Vuillemin cepa LBB-1 en condiciones de laboratorio. Fitosanidad 15: 51–57.

Ludwig, S.W. and R.D. Oetting. 2001. Susceptibility of natural enemies to infection by *Beauveria bassiana* and impact of insecticides on *Ipheseius degenerans* (Acari: Phytoseiidae). J. Agric. Urban Entomol. 18: 169–178.

Magalhaes, B.P., J.C. Lord, S.P. Wraight, R.A. Daoust and D.W. Roberts. 1988. Pathogenicity of *Beauveria bassiana* and *Zoophthora radicans* to the coccinellid predators *Coleomegilla maculata* and *Eriopsis connexa*. J. Invertebr. Pathol. 52: 471–473.

Mahdavi, V., M. Saber, H. Rafiee-Dastjerdi and A. Mehrvar. 2013. Susceptivility of the Hymenopteran parasitoid, *Habrobracon hebetor* (Say) (Braconidae) to the entomopathogenic fungi *Beauveria bassiana* Vuillemin and *Metarhizium anisopliae* Sorokin. J. J. Biol. Scien. 6: 17–20.

McGuire, M.R., M. Ulloa, Y.H. Park and N. Hudson. 2006. Biological and molecular characteristics of *Beauveria bassiana* isolates from California *Lygus hesperus* (Hemiptera: Miridae) populations. Biol. Contr. 33: 307–314.

Musser. F., J. Robbins, G. Cook, G. Studebaker, J. Greene, E. Burris, R. Bagwell, S. Stewart, A. Catchot and G. Lorenz. 2007. Comparison of direct and indirect sampling

methods for tarnished plant bug (Hemiptera: Miridae) in flowering cotton. J. Econ. Entomol. 100: 1916–1923.

Phelps, J.B., J.T. Ruscoe and W.H. McCarty. 1997. Cotton development following early square removal, pp. 1412–1413. In: Proceeding, 1997 Beltwide Cotton Conferences. National Cotton Council, Memphis, TN.

Portilla, M., G.L. Snodgrass and D. Streett. 2011. Effect of modification of the NI artificial diet on the biological fitness parameters of mass reared western tarnished plant bug, *Lygus hesperus*. J. Ins. Sci. 11: 1–10.

Portilla, M., G.L. Snodgrass and R. Luttrell. 2014. Effect of morning and night application of Beauveria bassiana strains NI8 and GHA against the tarnished plant bug in cotton. Pp. 729–734. In: Proceedings, Beltwide Cotton Conferences. National Cotton Council, Memphis, TN.

Portilla, M., G.L. Snodgrass and R. Luttrell. 2014a. A novel bioassay to evaluate the potential of *Beauveria bassiana* strain NI8 and the insect growth regulator novaluron against *Lygus lineolaris* on a non-autoclaved solid artificial diet. J. Ins. Sci. 14: 1–13.

Portilla, M., J. Walker, O. Perera, N. Seiter and J. Greene. 2016. Estimation of median lethal concentration of three isolates of *Beuaveria bassiana* for control of *Megacopta cribaria* (Heteroptera: Plataspidae) bioassayed on solid *Lygus* spp. diet. Insects 7 : 1–13.

Robbins, J.T., G.L. Snodgrass and F.A. Harris. 2000. A review of wild host plants and their management for control of the tarnished plant bug in cotton in the southern U.S. Southwest. Entomol. 23, 21–25.

Rossoni, C., S.O. Kassab, E.S. Loureiro, F.F. Pereira, D.P. Costa, R.H. Barbosa and J.C. Zanuncio. 2014. *Metarhizium anisopliae* and *Beauveria bassiana* (Hypocreales: Clavicipitaceae) are compatible with *Cotesia flavipes* (Hymenoptera: Braconidae). Florida Entomol. 97: 1794–1804.

Roy, H. E. and E. Cotrell. 2008. Forgotten natural enemies: Interaction between coccinellids and insect-parasitic fungi. Eur. J. Entomol. 105: 391–398.

Scott, W.P. and G.L. Snodgrass. 2000. A review of chemical control of the tarnished plant bug in cotton. Southwestern Entomol. Suppl. 23: 67–81.

Snodgrass, G.L. 1991. *Deraecoris [sic] nebulosus* (Heteroptera: Miridae) little known predator in cotton in the Mississippi Delta. Fla. Entomol. 74: 340–344.

Snodgrass, G.L. 1996. Pyrethroid resistance in field population of tarnished plant bug (Heteroptera: Miridae) in cotton in the Mississippi Delta. J. Econ. Entomol. 89: 783–790.

Snodgrass, G.L., J. Gore, C.A. Abel and R. Jackson 2009. Acephate resistance in population of the tarnished plant bug (Heteroptera: Miridae) from the Mississippi River Delta. J. Econ. Entomol. 102: 699–707.

Snodgrass, G.L. and W.P. Scott. 2000. Seasonal changes in pyrethroid resistance in tarnished plant bug (Heteroptera: Miridae) population during a three-year period in the delta area of Arkansas, Louisiana, and Mississippi. J. Econ. Entomol. 93: 441–446.

SAS. **2013**. SAS/STAT user's manual, version 9.4 ed. SAS Institute, Cary, NC.

Tanada, Y. and H.K. Kaya. 1993. Insect Pathology. Academic Press. San Diego, CA. 666 pp.

Thungrabeab, M. and S. Tongma. 2007. Effect of entomopathogenic fungi, *Beauveria bassiana* (Balsam) and *Metarhizium anisopliae* (Metsch) on non target insects. Sci. Tech. J. 7: 8–12.

Todovora, S.I., J.C. Cote and D. Coderre. 1996. Evaluation of the effects of two *Beauveria bassiana* (Balsamo) Vuillemin strains on the development of *Coleomegilla maculata lengi* Timberlake (Col., Coccinellidae). J. Appl. Entomol. 120: 159–163.

Ugine, T.A. 2012. Developmental times and age-specific life tables for *Lygus lineolaris* (Heteroptera: Miridae) reared at multiple constant temperatures. Environ. Entomol. 41: 1–10.

Velez, P.E., F.J. Posada, P. Marin, A. Bustillo, M.T. Gonzales and E. Osorio. 1997. Tecnicas para el control de calidad de formulaciones de hongos entomopatogenos. *Boletin Tecnico Cenicafe* 17: 37.

Young, O.P., T.C. Lockley and G.B. Edwards. 1989. Spiders of Washington County, Mississippi. J. Arachnol. 17: 27–41.

Zhu, Y.C., J. Adamczk, T. Rinderer, J. Yao, R. Danka, R. Luttrell and J. Gore. 2015. Spray toxicity and risk potential of 42 commonly used formulation of row crop pesticides to adult honey bees (Hymenoptera: Aphidae). J. Econ. Entomol. 108: 2640–2647.