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Research

Laboratory and Field Investigations on Compatibility of *Beauveria bassiana* (Hypocreales: Clavicipitaceae) Spores With a Sprayable Bioplastic Formulation for Application in the Biocontrol of Tarnished Plant Bug in Cotton

Maribel Portilla,[1,4] Hamed K. Abbas,[2] Cesare Accinelli,[3] and Randall Luttrell[1]

[1]USDA-ARS Southern Insect Management Research Unit, 141 Experiment Station Road, P.O. Box 346, 141 Experiment Station Road, Stoneville, MS 38776, [2]USDA-ARS National Biological Control Laboratory, 59 Lee Road, Stoneville, MS 38776, [3]Department of Agricultural and Food Sciences, Alma Mater Studiorum—University of Bologna, Bologna 40127, Italy, and [4]Corresponding author, e-mail: maribel.portilla@ars.usda.gov

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Abstract

Two isolates of *Beauveria bassiana* (Balsamo) Vuillemin, including the commercial strain GHA and the Mississippi Delta native NI8 strain, and two emulsifiers, Tween-80 and a starch-based sprayable bioplastic, were evaluated in the laboratory and field for pathogenicity and infectivity against the tarnished plant bug, *Lygus lineolaris* (Palisot de Beauvois) (Heteroptera: Miridae). The effect on fruit damage based on within-season cotton plant mapping was also examined. The highest mortality 10 d after treatment was found with insects caged on cotton terminals sprayed with NI8 + Tween-80, followed by those exposed to NI8 + bioplastic. Similarly, sporulation was shown to be higher in NI8 + Tween-80 than in other treatments. Plots sprayed with *B. bassiana* showed at least a twofold decrease in tarnished plant bug adults 3 d after treatment compared with control plots. Little to no variation was observed in tarnished plant bug nymph populations between treated and untreated plots. Within-season plant mapping provided clear evidence of damage to cotton caused by tarnished plant bug. The highest percentage retention of all first position fruiting structures was observed in plots treated with NI8 + Tween-80 (93.41 ± 1.51) followed by NI8 + bioplastic (90.25 ± 1.52). Both treatments were significantly different when compared with GHA + Tween-80 (82.89 ± 2.26) and GHA + bioplastic (70.48 ± 3.19), and both GHA formulations did not differ from the control (63.61 ± 2.96). Overall, these results indicated that *B. bassiana* application resulted in >50% mortality of tarnished plant bug regardless of the isolates by direct spray or by contact. However, the superior performance of the Mississippi Delta native NI8 strain was observed in all treatment applications and evaluation times.

Key words: bioplastic, NI8, microbial control, *Lygus*

The tarnished plant bug, *Lygus lineolaris* (Palisot de Beauvois) (Heteroptera: Miridae), is a major pest of cotton in Mississippi and across the southern United States (Cook 2018). Tarnished plant bug became a higher priority pest following the eradication of the boll weevil, *Anthonomus grandis* (Boheman) (Coleoptera: Curculionidae), and the development of resistance in tarnished plant bug to several classes of common insecticides (Snodgrass 1996, Hollingsworth et al. 1997, Snodgrass and Scott 2002, Snodgrass et al. 2009, Parys et al. 2017, 2018). Cook (2018) noted that during the 2017 growing season, the *Lygus* complex caused more fruit loss cotton than any other insects, and tarnished plant bug alone infested >250,000 ha of cotton in 2017, resulting in a yield loss of >100,000 bales ($50 million). A survey conducted in Mississippi in 2008 indicated that both the number of acres scouted and the amount of insecticide used on cotton doubled when compared with four previous years. The increase in insecticide use (68%) was attributed largely to increased problems with tarnished plant bug control (Musser et al. 2009). Portilla et al. (2017) noted that since the late 1990s, there has been keen interest in decreased reliance on chemical insecticides for control of this insect pest, including efforts to find naturally occurring microbial pathogens that can be used for biological control.

These alternative methods of control include the use of the entomopathogenic fungus *Beauveria bassiana* (Balsamo) Vuillemin. This entomopathogenic fungus is a cosmopolitan pathogen found in almost all ecosystems and is one of the most commonly

applied mycoinsecticides for a variety of insect control purposes (Kimtova and Bajan 1982, Hajek et al. 1987, Todovora et al. 1996, Thungrabeab and Tongma 2007, Leyva et al. 2011, Rossini et al. 2014). Mahdavi et al. (2013) mentioned that commercial mycoinsecticides could regulate insect populations through inundative and inoculative application. The GHA strain (BotaniGard 22WP) is one of the commercial mycoinsecticides currently registered by the U.S. Environmental Protection Agency. In 2002, an isolate of *B. bassiana* was found naturally infecting tarnished plant bug in the Mississippi Delta. It was referred to as either NI8 or TPB 3 strain (Leland and Snodgrass 2005). A number of different studies using this strain provided encouraging results because of its higher activity against tarnished plant bug, and additional research has been initiated for the use of NI8 as an alternative tarnished plant bug control measure. The native Mississippi Delta NI8 strain appears to be a promising isolate for control of tarnished plant bug in this important cotton-growing area based on comparative toxicity to other commercial *B. bassiana* strains such as GHA (i.e., lower LC_{50}, higher infection, and increased spore production; Leland 2005, Leland and Snodgrass 2005, Leland et al. 2005, McGuire et al. 2005, Ugine 2012, Portilla et al. 2014a). *Beauveria bassiana* is usually applied in the form of spores, which need stabilizing agents to facilitate application, stability, and enhancement of activity. Oil-based emulsion formulations are superior spray carriers with increased probability of direct contact between fungal spores and host insects. Due to its emulsifying action, Tween-80 has been shown to enhance the production of enzymes such as cellulose, amylase, and lignase (Mishra et al. 2013). It has also been shown to be an activator of fungal spores, making it indispensable for laboratory bioassays and field trials (Jin et al. 2008). More recent studies have explored the feasibility of using a bioplastic-based formulation as a carrier of microbiological agents, including atoxigenic isolates of *Aspergillus flavus* (Link) (Eurotiomycetes: Eurotiales) and *Trichoderma* spp. (Accinelli and Abbas 2011). Therefore, this study was carried out under laboratory and field conditions to evaluate the feasibility of a sprayable bioplastic formulation for carrying spores of the *B. bassiana* NI8 strain and the commercial GHA strain, in the biocontrol of tarnished plant bug in cotton.

Materials and Methods

Insect Colony

Adult tarnished plant bug were obtained from a colony established in 1998 at the USDA-ARS Biological Control Rearing and Research Unit in Starkville, MS (Cohen 2000, Portilla et al. 2017). The adults were reared following the procedure described in Portilla et al. (2011), which was specifically designed for mass production of even-aged individuals. Insects were held in environmental chambers with a photoperiod of 12:12 (L: D) h, 27°C, and 60% RH. Mixed-sex adults that were 1–2 d old were used for bioassays.

Bioassay Procedure to Determine Bioplastic Concentration and Spore Compatibility for the NI8 Strain

NI8 spore powder (technical powder) was produced at the USDA-ARS, National Biological Control Laboratory, Stoneville, MS, using a small-scale biphasic culture system for solid-substrate fermentation as described by Portilla et al. (2016). Harvested spore powder from the NI8 strain was assessed for spore germination and spore quantification (spores/mm^2; Portilla et al. 2017).

The sprayable bioplastic formulation was obtained by dispersing bioplastic granules in water as described in Accinelli et al. (2015). Briefly, starch-based granules were moistened with water (80% wt/vol) and allowed to stand for approximately 2 h at room temperature and then homogenized by stirring at 80°C for 2 h in water (20% wt/vol). After blending for 15 min, the bioplastic dispersion was passed through a 1-mm sieve, diluted to 1% in water, and stored at 4°C until used. Then, 0.5 g of harvested spore powder that contained 1.2×10^{11} spores/g was suspended in 50 ml of bioplastic formulation prepared with increasing percentages of bioplastic (0.25, 0.50, 0.75, and 1% wt/vol). The same spore powder was dispersed in 0.04% Tween-80 (TWEEN 80; Sigma–Aldrich P8074, Darmstadt, Germany) or water. Stock spore suspensions of the NI8 strain were diluted to obtain a concentration of 7×10^7 spores/ml (spores 97% viability). Aliquots of 6 ml of each spore suspension (NI8 + bioplastic concentration) and water control (no spores) were applied to each of three replicate groups (group per concentration) of 30 mixed-sex adults of tarnished plant bug that were 2–3 d old. Suspensions were applied using a specially designed spray tower that covered an area of 38.5 cm diameter (arena) fitted with an air-atomizing nozzle 1/4J with a fluid cap 2850 and air cap 70 (Spraying System Co. FN5925-001-001A, Wheaton, IL; Portilla et al. 2017). To avoid cross-contamination between sprays, treatments were applied from lowest to highest concentrations, and nozzles were changed for each concentration. Spray from each treatment and replications covered a circular area of 35.81 cm in diameter in which the group of insects was placed. After each application, adults were released into an insect observation cage (30.5 cm^3; BioQuip 1466A, Los Angeles, CA; a cage per each rep and per each treatment) to let them dry from exposure to the atomized spray. The sprayed insects were then placed individually into 37-ml plastic cups (T-125 SOLO-cup, Pleasant Prairie, WI) containing solid diet. Adults were examined daily for mortality. Dead insects were kept individually in the same cup and were daily checked for sporulation of the fungus inside the dead insect body. Adults of tarnished plant bug were held for 10 d in an environmental room at 27°C, 65% RH, and photoperiod of 12:12 (L:D) h.

Bioassay Procedure for Infectivity by NI8 and GHA Strains by Contact

A screening assay was carried out using two different strains of *B. bassiana* (the native NI8 strain and the commercial GHA strain). The commercial GHA strain (BotaniGard 22WP) was obtained from LAM International Corporation, Butte, Montana, and used according to labeled instructions. Spore powder of NI8 and GHA strains were mixed in water containing 0.4 ml of Tween-80/L and 1% concentration of bioplastic (75 l/ha), respectively. Both strains were diluted to obtain a concentration of 2.47×10^{12} spores/ha. Twenty-five plots (0.028 ha each) were planted with eight rows (15.24 m in length) of cotton (101.6 cm wide), *Gossypium hirsutum* L. (Malvales: Malvaceae) (DP1321B2RF Bollgard II, Delta and Pine Land Company, Scott, MS) in late May 2013. The cotton plots were flanked by four rows of mustard, *Brassica juncea* L. (Brassicales: Brassicaceae) (Indian Florida Broadleaf Mustard, L911516, The Wax Company, LLC, Amory, MS) and four rows of corn, *Zea mays* L. (VT2Pro corn, DKC66-97, DeKalb Genetics Corp., DeKalb, IL) were planted in February and March of 2013, respectively. Mustard was planted as an early attractant to build tarnished plant bug population densities and corn was planted as a barrier to avoid cross-contamination among plots and strains. The tarnished plant bug population was sampled from the mustard

biweekly after flowering (10 sweep net samples per plot). Once the tarnished plant bug population built to approximately 5–10 adults per 10 sweep net samples on mustard, it was mowed to facilitate insect movement onto adjacent cotton plots. Cotton plots were sprayed with a John Deere 6000 sprayer (Moline, IL) equipped with a ten multiboom spray system (BellSpray Inc., Opelousas, LA) with hollow cone nozzles (TX-VS12, Spraying System Co., Glendale Height, IL). The sprayer was calibrated to deliver 112.3 l/ha water and plots were sprayed on July 2013 with the *B. bassiana* strains or water alone as a control (five plots per strain treatments). The multiboom sprayer allows for the application of multiple treatments with minimal cross-contamination. One hour after *B. bassiana* application, three terminals (top three nodes of cotton plant) per plot were cut and placed under laboratory conditions individually in a Pop-Up Butterfly Release Cage, BC710 (Educational Caterpillar Supply, Trussville, AL). Seventy-five terminals were used in the 25 plot study (three cages per plot, 15 terminals per treatment). Thirty tarnished plant bug adults of 2 d old from a laboratory colony were released in each cage. Cages with cotton terminals and released insects were kept in an environmental room at 27°C, 65% RH, and a photoperiod of 12:12 (L:D) h. After insects were released, cages were gently shaken to allow the insects to be in contact with the cotton foliage surface. Adults were collected 24 h after release. Insects from the cages were then individually moved to a solo cup with solid diet (Portilla et al. 2014b). Dead insects were kept in the same cup and were checked daily for fungal sporulation. Adult tarnished plant bug was held in an environmental room at 27°C, 65% RH, and photoperiod of 12:12 (L:D) h and checked daily for insect mortality for 10 d.

Effect of NI8 and GHA Strains on Mortality of Tarnished Plant Bug Populations Under Field Conditions and Fruit Damage Based on the Within-Season Yield Cotton Plant Mapping

This experiment was conducted at Stoneville, MS, in the previously described cotton plots. High numbers of tarnished plant bug on mustard moved to cotton plots following mowing. Mustard was mowed on 27 June 2013, and cotton plots were sprayed (see Bioassay Procedure for Infectivity by NI8 and GHA Strains by Contact) on 8 July 2013. Sampling of tarnished plant bug populations was conducted 1 wk prior to and two consecutive weeks following the *B. bassiana* spray. Sampling consisted of 10 sweep net samples per plot per replication, and the number of nymphs and adults present was recorded. All insects were indexed by date of sample. In addition to the sampling population, within-season yield mapping was performed on 23 July 2013 to observe the effects of tarnished plant bug feeding on cotton fruit retention. Plant mapping was performed on 10 plants per plot per replication (250 plants total). Data recorded on selected plant mapping date included total nodes, total fruiting nodes, first fruiting node, nodes above white flowers, first position squares, first position flower or boll, first position open bolls, first position damage fruit, missing first position fruit, first position flowers and bolls, total first position fruits, number flowers/boll nodes, percentage retention top 3 fruiting position, percentage retention top 5 fruiting position, percentage retention nodes above white flowers, and percentage retention of all first position site including all undamaged squares, flowers, and bolls.

Statistical Analysis

All experiments were analyzed using SAS 9.4 (SAS Institute 2013). A randomized complete block design with factorial arrangements (treatment concentrations × replicates) was used for each laboratory and field experiment: 6 × 3 (laboratory compatibility/evaluation time) and 5 × 5 (direct spray/evaluation time and variables for plant mapping). Mortality, sporulation under laboratory and field conditions, tarnished plant bug field population density, and plant mapping variables were analyzed by using a one-way ANOVA followed by Tukey's HSD test.

Results

Bioplastic Concentration for Spore Compatibility for NI8 Strain

There were low significant differences in tarnished plant bug mortality 3 d after spraying insects in the laboratory ($F = 2.27$; df = 5, 539; $P = 0.0464$). No mortality was found 3 d after spraying with water and Tween-80. No significant differences in mortality were found 5 d after spraying with the sprayable bioplastic formulation containing 1% bioplastic (80.0 ± 0.4) when compared with Tween-80 (75.5 ± 0.4). However, both were significantly greater than that observed with bioplastic 0.5% alone (54.4 ± 0.5), 0.75% alone (46.6 ± 0.5), and 0.25% alone (40.0 ± 0.4; $F = 37.32$; df = 5, 539; $P \leq 0.0001$). No mortality of tarnished plant bug was observed in the water alone control (0% mortality). Ten days after spraying, a mortality of 97.7% of tarnished plant bug was found in tarnished plant bug sprayed with bioplastic and Tween-80 followed by 84.4, 83.3, and 66.6% for 0.5, 0.75, and 0.25% concentration, respectively ($F = 106.3$; df = 5, 539; $P \leq 0.0001$). No mortality was found in the control group 10 d after spraying. Sporulation percentage was shown to be higher in Tween-80 (97.7%); however, it did not differ statistically when compared with 1% bioplastic (93.3%). No sporulation was found in the control group. Significantly lower levels of sporulation were found with the lower bioplastic concentrations of 0.5, 0.75, and 0.25% with 77.7, 76.6, and 63.3% sporulation, respectively ($F = 101.5$; df = 5, 539; $P \leq 0.0001$; Fig. 1).

Infectivity of Tarnished Plant Bug by NI8 and GHA Strain by Contact

No significant differences in mortality were found among treatment ($F = 1.39$; df = 4, 2,249; $P = 0.2409$) 3 d after exposure. However, significant differences were found 5 d after treatment ($F = 2.27$; df = 5, 539; $P \leq 0.0001$) in which tarnished plant bug exposed to the water alone control (5.33 ± 0.011) did not differ from those exposed to the standard strain GHA + bioplastic (4.88 ± 0.011) and GHA + Tween-80 (6.67 ± 0.012). However, significantly higher mortalities were observed for caged insects exposed to cotton branches sprayed with NI8 + bioplastic (20.22 ± 0.019) and NI8 + Tween-80 (14.89 ± 0.017). Mortality of tarnished plant bug in the water alone control 10 d after exposure was observed, but it was the lowest among treatments (10.22 ± 0.014; Fig. 2). The highest mortality 10 d after treatment was found with insects caged on cotton terminals sprayed with NI8 + Tween-80 (65.78 ± 0.02) followed by those exposed to NI8 + bioplastic (49.11 ± 0.02). Both were significantly greater than those observed with GHA-bioplastic (26.44 ± 0.011) and GHA-Tween-80 (29.78 ± 0.011; $F = 107.10$; df = 4, 2249; $P \leq 0.0001$). Similarly, sporulation was shown to be higher in NI8 + Tween-80 (54.00 ± 0.011) than in other treatments (40.66, 20.88, 10.44, and 4.89 for NI8 + bioplastic, GHA + Tween-80, GHA + bioplastic, and control, respectively; $F = 120.73$; df = 4, 2249; $P \leq 0.0001$). Low sporulation was found in the control group (4.89 ± 0.01), probably due to cross-contamination. The level observed did not differ from that found with GHA + bioplastic (10.44 ± 0.014).

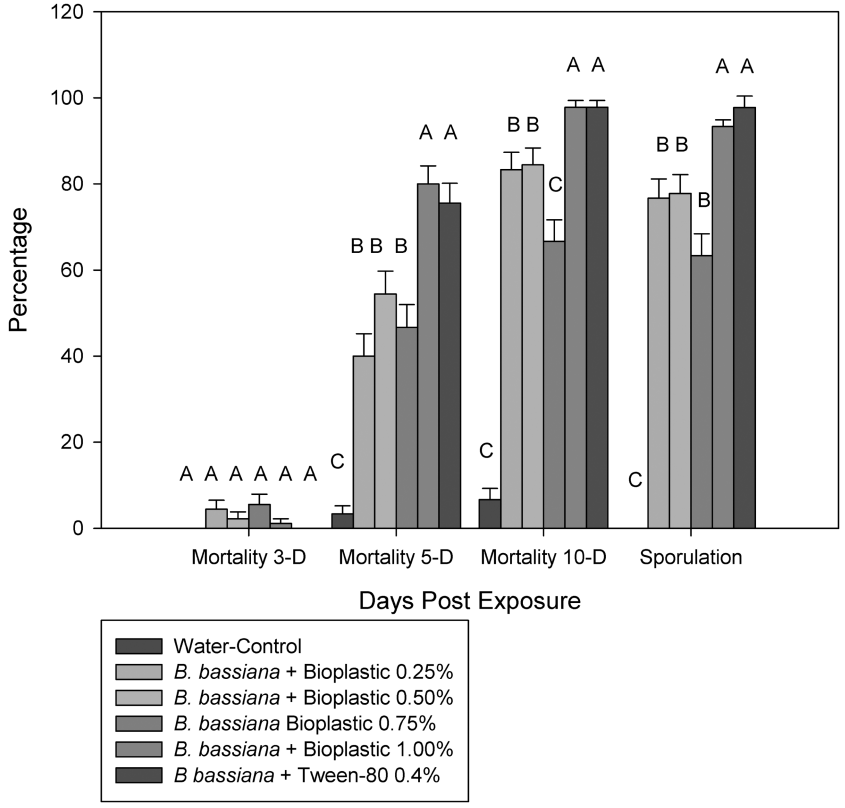

Fig. 1. Cumulative mortality and sporulation percentage of tarnished plant bug exposed to *Beauveria bassiana* (7×10^7) at four different concentrations of bioplastic under laboratory conditions. Insects were fed with solid diet after spray. Bars labeled with a different letter were significantly different at $P = 0.05$. Tukey's HSD test.

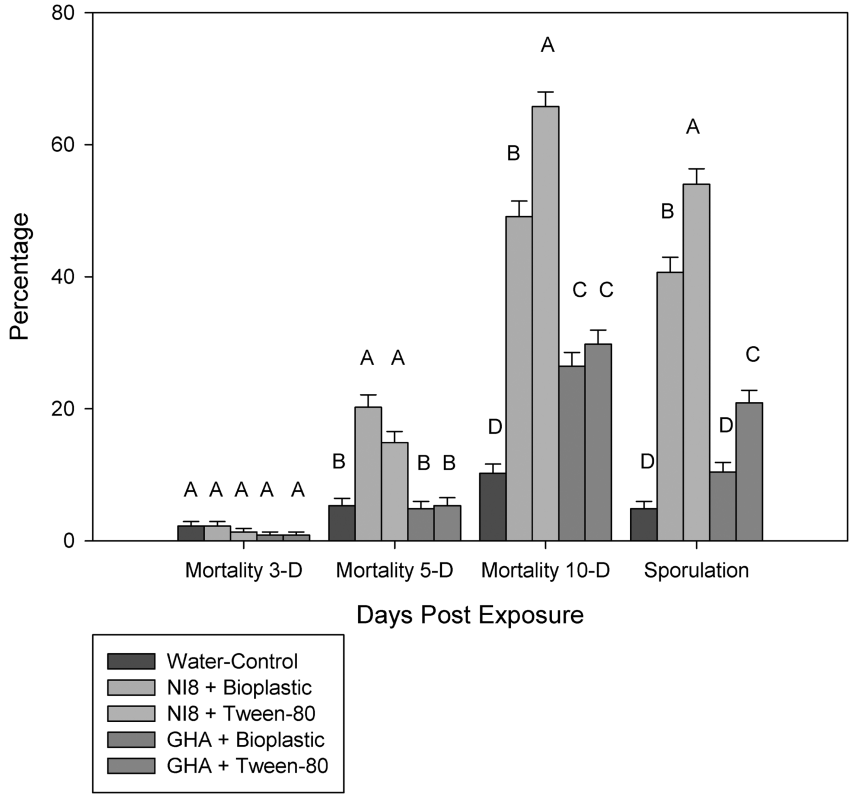

Fig. 2. Cumulative mortality and sporulation percentage of tarnished plant bug exposed to the native strain NI8 and the commercial strain GHA *Beauveria bassiana* (2.47×10^{12}/ha) applied on caged insects under field conditions. Insects were fed with artificial diet after spray. Bars labeled with a different letter were significantly different at $P = 0.05$. Tukey's HSD test.

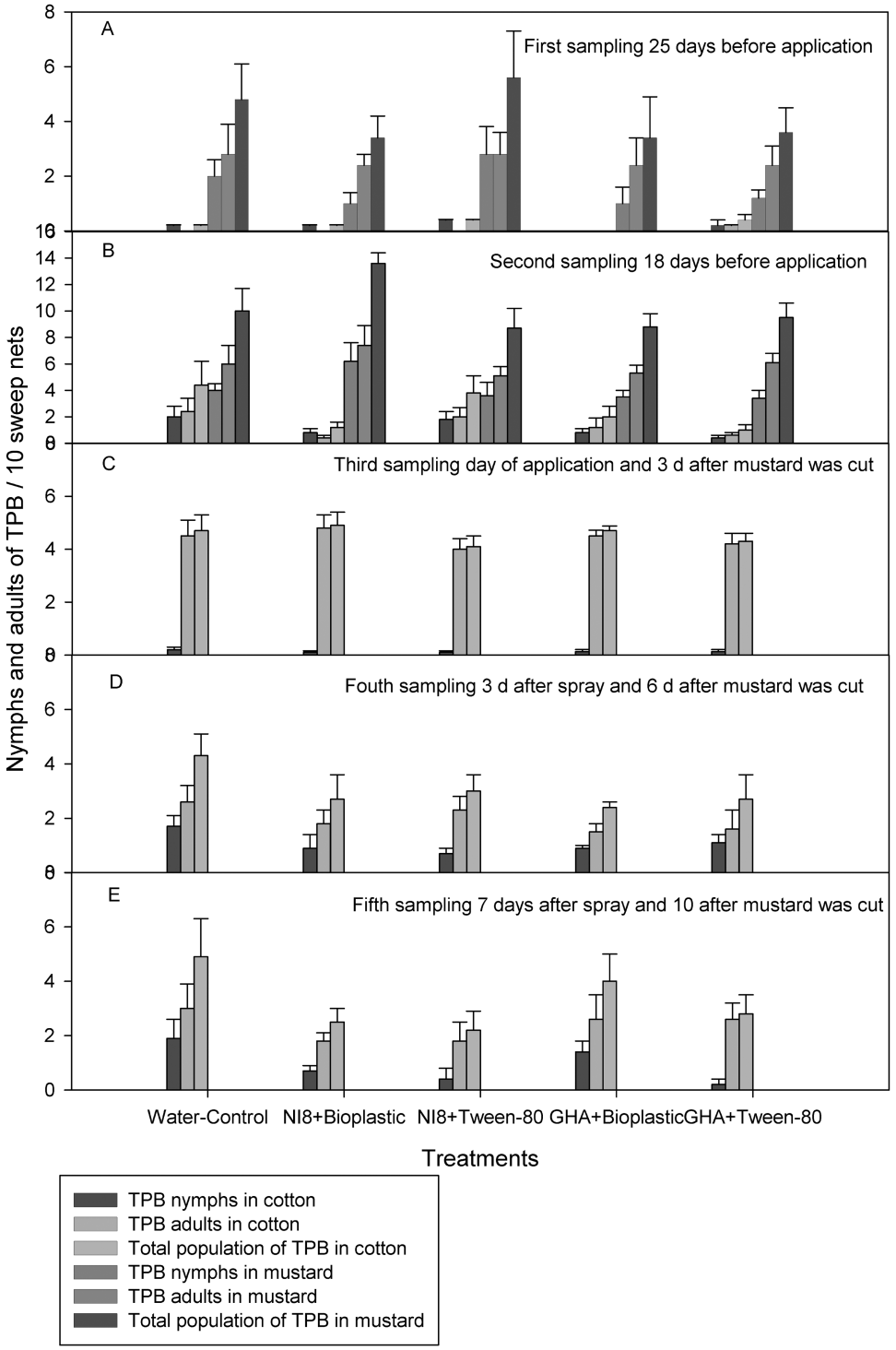

Fig. 3. Effect of the native strain NI8 and the commercial strain GHA *Beauveria bassiana* NI8 and GHA strains (2.47 × 10^{12}/ha) on tarnished plant bug population under field conditions.

Effect of NI8 and GHA Strains on Mortality of Tarnished Plant Bug Population Under Field Conditions and Cotton Fruit Retention

The numbers of tarnished plant bug observed on each sample date are shown in Fig. 3A–E. As expected, populations of nymphs and adults of tarnished plant bug on mustard grew faster than those in cotton plots with no significant differences between plot treatments (Fig. 3A and B; Table 1). Tarnished plant bug numbers increased twofold 7 d after the first sampling day in both cotton and mustard plots (Fig. 3B), with no significant differences among treatments. Fig. 3C displays threefold to fourfold increase in tarnished plant bug numbers 3 d after the mustard was mowed. No significant differences in the number of nymphs (F = 0.39; df = 8, 16; P = 0.8129), adults (F = 0.40; df = 8, 16; P = 0.8027), or total tarnished plant bug number (F = 0.49; df = 8, 16; P = 0.7458) per 10 sweeps per treatment plot were found. Although no significant differences were

found 3 d after treatment, plots sprayed with *B. bassiana* showed higher tarnished plant bug mortality in adults than the control (Fig. 3E), which was sprayed with water only. Numbers of tarnished plant bug adults (individuals per 10 sweeps) in plot sprayed with NI8 + Tween-80 (2.20 ± 0.7), NI8 + bioplastic (2.50 ± 0.5), and GHA-Tween-80 (2.80 ± 0.75) remained lower 7 d after treatments. In the GHA-bioplastic treatment (4.00 ± 1.40), population densities were similar to those in the water control (4.90 ± 1.10; Fig. 3D). Little variation in the number of nymphs was observed among plots, suggesting that *B. bassiana* neither NI8 nor GHA, regardless the addition of emulsifier Tween-80 or bioplastic, will affect population of immature stages of tarnished plant bug.

The within-season mapping of cotton fruit retention and the effect of *B. bassiana* treatments on numbers of all first position undamaged squares, flowers, bolls, and percentage of nodes above white flowers are presented in Table 2. There were statistically significant differences in most of the parameters measured, including missing first position fruit ($F = 0.89$; df = 8, 241; $P \leq 0.0001$), percentage retention top 3 fruit position ($F = 29.58$; df = 8, 241; $P \leq 0.0001$), percentage retention top 5 fruit position ($F = 37.20$; df = 8, 241; $P \leq 0.0001$), percentage retention nodes above white flowers ($F = 28.87$; df = 8, 241; $P \leq 0.0001$), and percentage retention of all first position ($F = 30.56$; df = 8, 241; $P \leq 0.0001$). In general, the highest percentage retention of all first position fruiting sites were those in plots treated with NI8 + Tween-80 (93.41 ± 1.51) followed by NI8 + bioplastic (90.25 ± 1.52). Both NI8 treatments had more fruit retention than plots treated with GHA-Tween-80 (82.89 ± 2.26) and GHA-bioplastic (70.48 ± 3.19). Although both GHA treatments had numerically higher fruit retention than the water-control treatment (63.61 ± 2.96), the differences were not significant ($P = 0.05$).

Discussion

Experimental work with the deutoromycete fungus *B. bassiana* has been conducted frequently with the surfactant Tween-80 or Sylwet L77 (Leland 2005, Leland and Snodgrass 2005, Leland et al. 2005, McGuire et al. 2005, Ugine 2012, Portilla et al. 2014b, Portilla et al. 2017). Nothing has been documented about the infectivity of *B. bassiana* and the spore compatibility of starch-based sprayable bioplastic formulation to control tarnished plant bug populations.

Table 1. Overall general lineal model of the effect of *Beauveria bassiana* NI8 and GHA strains on tarnished plant bug population under field conditions (Fig. 3)

Tarnished plant bug per 10 sweep nets	Sprays				
	First	Second	Third	Fourth	Fifth
Nymphs in cotton	$P = 0.736$, $F = 0.50$	$P = 0.081$, $F = 2.53$	$P = 0.813$, $F = 0.40$	$P = 0.844$, $F = 0.34$	$P = 0.219$, $F = 1.61$
Adults in cotton	$P = 0.436$, $F = 1.00$	$P = 0.140$, $F = 2.02$	$P = 0.803$, $F = 0.40$	$P = 0.740$, $F = 0.50$	$P = 0.851$, $F = 0.33$
Total tarnished plant bug in cotton	$P = 0.634$, $F = 0.65$	$P = 0.052$, $F = 2.96$	$P = 0.746$, $F = 0.50$	$P = 0.955$, $F = 0.16$	$P = 0.576$, $F = 0.74$
Nymphs in mustard	$P = 0.251$, $F = 1.49$	$P = 0.289$, $F = 1.37$	—	—	—
Adults in mustard	$P = 0.991$, $F = 0.07$	$P = 0.994$, $F = 1.72$	—	—	—
Total tarnished plant bug in mustard	$P = 0.645$, $F = 0.63$	$P = 0.023$, $F = 3.80$	—	—	—

(—) Not available.
df = 4, 24 for all variables.

Table 2. Effect of *Beauveria bassiana* NI8 and GHA strains with sprayable bioplastic formulation under field conditions on tarnished plant bug population and the damage of fruits based on within-season yield cotton plant mapping

Indices of tarnished plant bug damage	Treatments (means ± SE)				
	Water control	NI8 + bioplastic	NI8 + Tween-80	GHA + bioplastic	GHA + Tween-80
Total nodes	14.26 ± 0.43a	15.06 ± 0.43a	15.00 ± 0.39a	15.28 ± 0.32a	15.44 ± 0.30a
Total fruiting nodes	9.12 ± 0.42b	10.82 ± 0.57ab	10.06 ± 0.38ab	10.22 ± 0.41ab	10.04 ± 0.35a
First fruiting nodes	5.14 ± 0.21ab	4.24 ± 0.33b	4.94 ± 0.19b	5.06 ± 0.21b	5.40 ± 0.25a
Nodes above white flowers	5.56 ± 0.31b	6.98 ± 0.35a	6.48 ± 0.33a	6.72 ± 0.28a	7.02 ± 0.46a
First position square	2.94 ± 0.27c	5.68 ± 0.19a	5.42 ± 0.18ab	3.86 ± 0.28bc	5.12 ± 0.18ab
First position flowers or bolls	2.82 ± 0.23b	3.92 ± 0.36a	4.02 ± 0.33a	2.86 ± 0.22b	3.24 ± 0.30ab
First position damaged fruits	0.02 ± 0.02a	0a	0.02 ± 0.02a	0.04 ± 0.02a	0a
Missing first position fruits	3.36 ± 0.31a	1.22 ± 0.19b	0.68 ± 0.14b	3.24 ± 0.38a	1.68 ± 0.23b
First position flowers and bolls	2.82 ± 0.23b	3.92 ± 0.36ab	3.96 ± 0.33a	3.12 ± 0.22ab	3.24 ± 0.30ab
Total first position fruits	5.76 ± 0.40c	9.60 ± 0.46a	9.38 ± 0.37a	6.98 ± 0.38bc	8.36 ± 0.37ab
Number squaring nodes	6.22 ± 0.31a	6.98 ± 0.35a	6.48 ± 0.33a	6.72 ± 0.28a	7.02 ± 0.46a
Number of flowers per boll nodes	3.32 ± 0.29a	4.84 ± 0.47a	4.28 ± 0.36a	4.00 ± 0.32a	4.08 ± 0.36a
Retention top 3 fruiting position (%)[a]	66.66 ± 3.81b	98.00 ± 1.13a	98.66 ± 0.93a	76.00 ± 3.30b	90.00 ± 2.56a
Retention top 5 fruiting position (%)[a]	57.20 ± 3.88b	95.60 ± 1.74a	96.00 ± 1.89a	68.40 ± 3.62b	88.80 ± 2.81a
Retention nodes above white flowers (%)	47.57 ± 3.67b	86.89 ± 3.04a	88.99 ± 2.84a	57.88 ± 4.21b	81.00 ± 3.58a
Retention all first position (%)[b]	63.61 ± 2.96c	90.25 ± 1.51ab	93.41 ± 1.51a	70.48 ± 3.19c	82.89 ± 2.26c

Means ± SE followed by the same letter in each row are not significantly different ($P < 0.05$ Tukey's HSD test).
[a]With undamaged squares.
[b]Include all undamaged squares, flowers, and bolls.

Accinelli and Abbas (2011) and Abbas et al. (2017) have demonstrated that biological control of aflatoxin contamination in corn using bioplastic formulation of *A. flavus* biocontrol strains could optimize application strategies. Field experiments conducted in Mississippi (United States) and Italy have shown that application of 1% bioplastic formulation with *A. flavus* strain K49 resulted in an average 96.5% reduction in aflatoxin contamination of harvested corn kernels relative to untreated control plots (Accinelli et al. 2009, 2015). Similar results were observed in our study under laboratory conditions where the sporulation and mortality percentage of 1% concentration of bioplastic + NI8 strain formulation (97.7 ± 0.02 and 93.3 ± 0.03, respectively) was as effective as the combination of the commercial surfactant Tween-80 + NI8 formulation 0.04% water solution (97.7 ± 0.02 and 97.7 ± 0.02, respectively). No significant differences were found between these two combinations, suggesting that bioplastic could help to promote sporulation of *B. bassiana* as much as the commercial Tween-80 under laboratory conditions (Fig. 1). Accinelli et al. (2015) also corroborated this occurrence when used bioplastic and *A. flavus* strains to control aflatoxin contamination in corn. They explained that it could be due to acetylation of starch to <10% acetate substantially alters the properties of the starch, reducing wettability to some extent, but increasing adherence to cuticle-coated surfaces which provide nutrients to support the growth of the fungus *A. flavus*.

Adult tarnished plant bug from the laboratory colony that was exposed to sprayed cotton terminals exhibited lower mortality and sporulation (Fig. 2) than those treated with a direct application in the laboratory (Fig. 1). These results agree with findings from Inglis et al. (2001) where entomopathogenic Hyphomycetes infected their hosts primarily through the external cuticle and the number of conidia acquired by the host determined the probability of death. This process increases the propagation of conidia through sporogenesis. Mortality of tarnished plant bug and sporulation of *B. bassiana* by contact demonstrated the capacity of tarnished plant bug adult to acquire a lethal dose of conidia (asexual reproductive spores) or propagate the fungus by walking or feeding on infected cotton terminals. In this experiment, bioplastic was not as effective as the commercial surfactant Tween-80 where the highest mortality and sporulation was found on insects released in terminals sprayed with NI8 + Tween-80 that statistically differed from terminals sprayed with NI8 + bioplastic formulation. These results also differed from studies conducted by Abbas et al. (2017) who noted that corn leaf application of the sprayable biocontrol *A. flavus* bioplastic formulation resulted in an average 97.1% reduction in aflatoxin contamination of harvested corn kernels relative to unsprayed control plots. This could indicate that the bioplastic efficiency probably depends on the structure of the leaf's surface, and the bioplastic cuticle-coated adherence in cotton foliage is much lower than in corn foliage. This could also vary by cultivar within crop. Similar behavior was observed on cotton branches sprayed with the commercial GHA strain. However, GHA + bioplastic did not differ statistically from the control, which had a sporulation of 4.80 ± 1.10 due to cross-contamination (Fig. 2). In general, the capacity of tarnished plant bug adults to acquire spores by contact will assure more than 50% mortality after spraying *B. bassiana* NI8 strain, regardless of the surfactant used.

The effects of agroecosystem heterogeneity on insect population dynamics, dispersal, and habitat selection have important implications for pest management (Carriere et al. 2006). McGuire et al. (2006) mentioned that one approach to managing *Lygus* spp. in cotton is to identify those areas where *Lygus* spp. overwinter and/or build to large populations prior to moving to a crop where they affect economic damage. Our data suggest that planting mustard next to cotton was a good example of observed host preference and movement of tarnished plant bug to cotton after their host become unsuitable for feeding and/or reproduction (Fig. 3A–C). In the Mississippi Delta, tarnished plant bug populations develop on a wide range of wild and cultured host plants, which deteriorate with age during hot and dry summer months, at which time tarnished plant bug move to early squaring cotton (Snodgrass et al. 2000, 2005). Differences in tarnished plant bug nymph and adult populations did not occur among treatments before and after mustard was mowed; however, highly significant differences were found among crops during all evaluations. The number of tarnished plant bug ranged from 4.0 to 4.8 adults and from 0.1 to 0.2 nymphs per 10 sweep net samples per plot at the time of treatment application, including the controls. No significant differences were observed in nymphs and adults 3 d after spraying, even though the plots sprayed with *B. bassiana* exhibited >50% adult mortality, and adult numbers ranged from 1.6 to 2.3 per sample and plot. These results corroborate the findings by McGuire et al. (2006) whose data showed more than half the mortality of the tarnished plant bug adult population occurred 3 d after NI8 strain application. This indicates that conidia germination, penetration, and the infection process could occur within a shorter period of time in the field than could occur in the laboratory. However, it is important to mention that the longer period for mortality obtained in the laboratory could be occurred due to the quality of the laboratory insects, which are normally more active, bigger, and stronger than feral populations. Portilla et al. (2014b) previously reported that median lethal time that ranged from 4.0 to 8.4 d for tarnished plant bug adults and nymphs between *B. bassiana* exposure and mortality in the laboratory. Interestingly, both strains with the combination of bioplastic and Tween-80 were similarly effective on tarnished plant bug adults 3 d after spray, which are comparable to the results reported by McGuire et al. (2006). They found no significant differences among isolates, although adults of tarnished plant bug have shown to be more pathogenic to NI8 than they were to GHA under laboratory conditions (Leland et al. 2005, Portilla et al. 2014b). However, it is important to note that in this experiment the combinations of GHA + Tween-80 and GHA + bioplastic could not maintain low tarnished plant bug numbers 7 d after application, where the commercial GHA + bioplastic were similar to the water control (Fig. 3E). This may suggest that the ability to transmit horizontally is more limited in the commercial GHA strain compared with NI8, where tarnished plant bug numbers declined within 7 d after application. This seems that the microclimatic condition worked in favor of NI8 because this isolate is native to the area where this study was conducted and isolated from tarnished plant bug adult. Fargues and Remaudiere (1977) and Inglis et al. (2001) have suggested that isolates of *B. bassiana* obtained from the environment and host would be more effective in controlling the target pest than other isolates.

The limited ability of both NI8 and GHA strains to control population of tarnished plant bug nymphs was also evident as nymph numbers increased within 3 d after spray. This suggests that the majority of nymph in this study may have been eggs at the time of application. Other studies have suggested that it is very possible that the lower susceptibility of nymphs and their subsequent recruitment into the adult population could be affecting adult population estimates (McGuire 2002, Leland et al. 2005, McGuire et al. 2005, Portilla et al. 2014b). However, it is important to mention that plots sprayed with NI8 + Tween-80 and GHA + Tween-80 the nymph population decreased from 0.7 and 1.1 nymphs per 10 sweep net samples 3 d after spray, respectively, to 0.4 and 0.2 nymphs per 10 sweep net samples per plot 7 d after spray. This differed from

McGuire et al. (2006) who reported only less than 10% on nymph populations at 10 d post-treatment in plots spray with NI8 + Silwet L-77. Therefore, this indicates that the surfactant Tween-80 could have had a better response as a spore activator that could affect younger nymphs. However, still adult population estimations will be affected because the fungal treatment regardless the surfactant may not be sufficiently persistent on the foliage to infect nymphs hatching 7 d after treatment and also will be unable to infect eggs, which are oviposited into plant tissue.

Plant responses to insect feeding are fundamental to developing economic injury levels, which are a major component of integrated pest management (Pedigo 1989). It has long been observed that the cotton plant has the potential for tolerance and/or compensation for early fruit loss, depending on the subsequent management and environmental growing conditions (Danforth et al. 1990). Therefore, knowing when the compensation ability is no longer a consideration is important in developing dynamic threshold, which complicates our ability to understand the impact of insect damage at different times of the season (Wilson 1985). Jenkins and McCarty (1995) described a method to measure the contribution of each fruiting site of the cotton plant using end-season maps. In this investigation, it was obvious the relation of within-season plant mapping (Table 2) and the within-season insect scouting (Fig. 3A–E) where allowed to examine the relationship between insect densities and ultimate value of fruit loss within particular cohorts of fruit available at the time of infestation. Tarnished plant bug prefers to feed on smaller squares when available but may also inflict damage to various sized bolls. Thus, the assessment of retention including all undamaged squares, flowers, and bolls after treatment was the primary requirement for this part of the study and is presented in Table 1.

The number of plant bugs that moved into cotton after mustard was mowed resulted in negative impacts on surviving fruit, mainly in plots sprayed with GHA + bioplastic as evidenced by missing first position fruit, which did not differ from water control. The indices of tarnished plant bug damages differed among isolates and surfactants. The highest retention percentage of all first position was obtained in plots treated with NI8 + Tween-80 follow by NI8 + bioplastic with significant differences between them; however, both were significantly different among GHA and water control. Overall, the present study indicated slight to significant positive effect of surfactant on *B. bassiana* spore's viability and its impact on tarnished plant bug population in laboratory bioassays and field trials. The impact on tarnished plant bug numbers was more pronounced when Tween-80 was added to NI8 than when it was when added to GHA. None of the laboratory bioassays or the foliar-spray treatments with the commercial GHA strain applied with Tween-80 or bioplastic during this study provided a high level of tarnished plant bug control, which is in accordance with the findings from other studies with tarnished plant bug (McGuire 2002; Leland et al. 2005; McGuire et al. 2005, Portilla et al. 2014a,b, 2016) and other insect pests like the Colorado potato beetle, *Leptinotarsa decemlineata* (Say) (Wraight and Ramos 2002).

Differences in mortality and sporulation did not occur among surfactants when used with the native NI8 strain under laboratory conditions nor in the field when tarnished plant bug numbers were measured. However, NI8 significantly differed among surfactants relative to cotton plant damage, with only 3% difference in retention of all first position fruiting structures. The laboratory results found from this investigation are supported by the studies of Luz and Batagin (2005), Mishra et al. (2013), and Kollmann et al. (2003) who reported a higher germination of *B. bassiana* spores when used with Tween-80. Likewise, Silva et al. (2005) reported Tween-80 to be the best surfactant for growth and germination of *Metarhizium anisopliae* (Sorokin). However, in contrast to the present study, significant reduction of mycelia growth for *Chondrostereum purpureum* (Pouzar) and detrimental speed on germination for *M. anisopliae* was reported by Tanuja et al. (2010) and Polar et al. (2005), respectively. Taken together our results indicate that the variation in spore viability and germination is largely dependent on surfactant toxicity along with natural host cell membrane.

To the best of our knowledge, no studies have been conducted in the field to measure the effect of *B. bassiana* on cotton damage by tarnished plant bug populations depending on spore compatibility with surfactants. Therefore, no comparable results were available for this specific part of the study. However, enough studies (Leland and Snodgrass 2005; Leland et al. 2005; McGuire et al. 2005, Portilla et al. 2014b, 2016) corroborated with our results, suggesting that the native NI8 strain collected from the Mississippi Delta and isolated from tarnished plant bug adults could be recommended for the control of tarnished plant bug population. The superior performance of the Delta native NI8 strain in combination with Tween-80 or bioplastic was observed in all treatments applications and times of evaluation. The spore compatibility of bioplastic obtained in all treatments should make this product an attractive alternative for use with native *B. bassiana* for control of tarnished plant bug.

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