EFFECTS OF MORNING AND NIGHT APPLICATIONS OF *Beauveria bassiana* STRAINS NI8 AND GHA AGAINST THE TARNISHED PLANT BUG IN COTTON

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Abstract

The tarnished plant bug, (TPB), *Lygus lineolaris* (Palisot de Beauvois), (Hemiptera: Miridae) an important pest of cotton (*Gosssypium hirsutum* L.) found in the Mississippi Delta is naturally attacked by the entomopathogenic fungus *Beauveria bassiana* (Balsamo) Vueillemin. In this study, two isolates of *B. bassiana* including the commercial strain GHA and the Mississippi Delta native NI8 strain were evaluated in the field for pathogenicity and infectivity against TPB. Effects of application times and solar radiation on mortality and sporulation were evaluated. In order to evaluate pathogenicity by direct spray, two-d old TPB adults from a laboratory colony were placed in cages located on the top part of cotton plants in the field prior to spraying *B. bassiana* strains with a multi-sprayer tractor calibrated to deliver 6.5 x 10^{12} spores / acre. Detailed observations were made on the effect of solar radiation by releasing 2-d old TPB adults in cages with sprayed branches of cotton plants cut 0, 1, and 2 days after morning and night applications. Differences on mortality and sporulation on TPB exposed to sprayed cotton branched for 24 hours were significant among treatments. Mortality and sporulation drastically decreased from 1.7-fold by the next day to 5.6-fold by the second day after *B. bassiana* NI8 night application and a reduction of 1.5-fold by the next day to 8.2-fold by the second day in morning application. Less than 10% sporulation was found two days after *B. bassiana* application for both strains. Overall, these results indicated that *B. bassiana* application resulted in decreased survival of TPB regardless of the isolates by direct spray or by contact. However, the superior performance of the Delta native strain NI8 was observed in all treatments applications and times of evaluation. An important obstacle to the efficacy of each isolates is their inability to survive exposure to solar radiation, which affects the use of this entomopathogenic fungus for the control of the TPB. However, the >50% mortality of TPB adults obtained by direct spray or by contact should make this fungi an attractive alternative for TPB control.

Introduction

Tarnished plant bug, TPB, *Lygus lineolaris* (Palisot de Beauvois), (Hemiptera: Miridae) is an important insect pest for many crops in US, and a primary pest of cotton in the Mississippi Delta. Nymphs and adults feed on reproductive plants parts resulting in direct economic damage. TPB became a pest for cotton in the Mississippi Delta through developing resistance to some common insecticides (Snodgrass 1996, Hollingsworth et al. 1997, Snodgrass and Scott 2002, Snodgrass et al., 2009). Therefore, other alternatives to control this pest have been studied including the use of the entomopathogenic fungus *Beauveria bassiana* (Balsamo) Vuillemin (Hypocreales: Clavicipitaceae) (Snodgrass and Elzen 1994, Leland and Behle 2004, Leland and Snodgrass, 2005; Leland 2005; Leland et al., 2005, McGuire et al. 2006, Lund et al. 2006a, Lund et al. 2006b, Spurgeon 2010, Ugine 2011 and 2012, Portilla et al. 2013 and 2014 in press).

For more than two decades, the entomopathogenic fungus *B. bassiana* has been studied as a potential alternative method to control TPB in cotton. Investigations with the commercially available strain of *B. bassiana* have shown that this entomopathogenic fungus was moderately effective in reducing adult TPB populations in cotton (Snodgrass and Elzen 1994, Noma and Strickler 1999 and 2000, Lund et al. 2006a). In 2002, an isolate of *B. bassiana* strain NI8 was found naturally infecting TPB in Mississippi Delta (Leland and Snodgrass, 2005). A number of laboratory studies using this strain have produced more encouraging results and supported research for its use as an alternative of TPB control measure (Leland and Snodgrass, 2005; Leland 2005; Leland et al., 2005, McGuire et al., 2006, Lund et al. 2006b, Ugine 2012, Portilla et al. 2014 in press). Portilla et al. (2013) evaluated in the field two isolates of *B. bassiana* the commercial GHA strain and the NI8 strain for pathogenicity and infectivity against TPB, reporting that NI8 strain of *B. bassiana* was superior to the commercially-available isolate suggesting that a 50% reduction of adult populations of *L. lineolaris* may occur 10 days after spray using a spray concentration about 73 – fold lower than that of the commercial GHA strain. McGuire et al. (2006) demonstrated that NI8 showed high virulence in the field to *Lygus hesperus*, Knight (Hemiptera: Miridae) when compared against the strains from

California and the commercial GHA strain. Leland (2005) exposed several strains of *B. bassiana* including NI8 to artificial sunlight, concluding that all isolates were highly susceptible to the negative effects of artificial sunlight, only one isolate from Mississippi (NI8) germinated at 35°C and obtained faster conidia growth at 32°C in isolates from *Lygus spp*. than the growth obtained in GHA. Portilla et al. (unpublished data) also found that differences in mortality and sporulation on TPB exposed to sprayed cotton branched were significant among concentrations and TPB can be easily infected through contact. However, results showed that survival of *B. bassiana* spores in the environment decreased with higher temperatures (July and August) resulting in reduced efficacy from one day to the next. In general, several environmental factors affect the efficacy of the entomopathogenic fungus *B. bassiana*, but solar radiation is the most detrimental environmental-stress factor that limits the use of this entomopathogenic fungus for the control of the TPB. This study was carried out in order to determine the effect of morning and night application, as well as solar radiation of two strains of *B. bassiana* on pathogenicity and infectivity by direct spray and by contact against TPB.

Materials and Methods

Fungal Isolate

The NI8 isolate of *B. bassiana* was obtained from the collection of USDA-ARS-SIMRU (Stoneville, MS) and was produced in a biphasic culture system that simulated industrial scale production according to the method described for solid substrate fermentation of *B. bassiana* (Jaronski 2013). The commercial strain GHA, (wettable powder mycoinsecticide) (BotaniGard 22Wp) was obtained from Laverlam International Corporation and used as indicated in its attached booklet.

Bioassay Procedure for infectivity by direct spray

A screening assay was carried out using two different strains of *B. bassiana* (the native strain NI8 and the commercial strain GHA) to evaluate the effect of morning (7:00 am) and night (7:00 pm) application and the effect of solar radiation on the effectiveness against adults of TPB. Thirty plots (0.068 acre each) were planted with cotton on late May 2013 and sprayed (Cone-Tip Quick TXVS12) with a multi-sprayer tractor on July 2013 with a concentration of $10x10^{12}$ spores /acre of both *B. bassiana* strains and a control (5 plots/ strain treatments). All sprays included 1.5 ml of Tween-80 per gallon of spray. Thirty 2-d old TPB adults from laboratory colony were placed in cages located on the top part of the cotton plants in the field prior to spraying with the *B. bassiana* strains. A total of 90 cages were used in the 30 plots (3 cages / plot). Adults were collected immediately after sprayed by knocking down individually into a solo cup with solid diet (Portilla et al. 2014 (in press). Dead insects were kept in the same cup and were daily checked for sporulation. Adults TPB were held in an environmental room at 27ºC, 65% RH, and 12: 12 (L: D) h photoperiod and checked daily for ten days.

Bioassay Procedure for infectivity by contact

After *B. bassiana* application, three branches (top cotton plant) per plot were cut at day 0, 1 and 2 and placed under laboratory conditions individually in a Pop-Up Butterfly Release Cage, BC710. One hundred and fifty cotton plant branches were used in the 30 plots (3 cages / plot, 50 branches / treatment). Thirty 2-d old TPB adults from a laboratory colony were released in each cage. Cages with branches and released insects were kept in an environmental room at 27ºC, 65% RH, and 12: 12 (L: D) h photoperiod. 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 hours after release. Released adult from the cages were knocked down individually into a solo cup with solid diet (Portilla et al. 2014 (in press). Dead insects were kept in the same cup and were daily checked for sporulation. Adults TPB were held in an environmental room at 27ºC, 65% RH, and 12: 12 (L: D) h photoperiod and checked daily for ten days.

Statistical Analysis

The experiment was set up as a completely randomized design with a factorial arrangement for each individual spray 3 x 2 x 3 x 5 for mortality and sporulation (three treatments: NI8 strain, GHA strain and water (control); two and three evaluation times: application time (morning and night) and time after exposure Day-3 (D-3), Day-5 (D-5), and Day-10 (D-10). Each treatment combination was repeated five times. Statistics were performed using SAS system software (SAS Institute, 2001). Data for mortality and sporulation was analyzed using the PROC GLM procedure to detect differences between treatments.

Results and Discussion

Results of both bioassays indicated that *B. bassiana* reduced TPB population regardless of the isolate, either by direct application or by contact. However, the Delta native strain NI8 was observed to have superior performance in all treatment applications and times of evaluation. Control mortality of < 10% and sporulation <5% by day 10 was found in both essays, which were significantly lower when compeered among time of applications and isolates (direct spray D-0: mortality F = 70.56; df = 11, 2688, P = 0.0001; sporulation F = 62.63 df = 11, 2688, P = 0.0001 Fig 1), (contact D-0: mortality F = 66.69; df = 11, 2688, P = 0.0001; sporulation F = 66.69; df = 11, 2688, P = 0.0001 Fig 2), (contact D-1: mortality F = 19.65; df = 11, 2688, P = 0.0001; sporulation F = 16.98; df = 11, 2688, P = 0.0001 Fig 3), (contact D-2: mortality F = 10.27; df = 11, 2688, P = 0.0001; sporulation F = 10.85; df = 11, 2688, P = 0.0001 Fig 4). Mortality and sporulation by direct spray or by contact seem to interfere at D-0 regardless of morning and night application of both strains. Higher mortality and sporulation by day 10 were obtained by direct spray (morning NI8: 65.7 ± SE 2.2, 54 ± SE 2.4, respectively; night NI8: 62.6 ± SE 2.2, 51.3 ± SE 2.3, respectively) than by contact (morning NI8: 54 ± SE 3.2, 42.6 ± 2.3, respectively; night NI8: 60 ± SE 2.1, 50.8 ± SE 2.7, respectively). No significant differences were found in mortality for morning and night applications within NI8, GHA, and Control. However, higher sporulation was obtained during night applications in NI8 and GHA strains when the infectivity was by contact. It seems that nocturnal applications may play an important role in creating a microhabitat in which *B. bassiana* can improve its viability. However, is very clear that mortality and sporulation drastically decreased from 1.7-fold by the next day to 5.6-fold by the second day after *B. bassiana* NI8 night application and a reduction of 1.5-fold by the next day to 8.2-fold by the second day in morning application. Less than 10% of sporulation was found two days after *B. bassiana* application for both strains. The survival of conidia deposited on substrates exposed to direct solar radiation is highly affected, but the mortality obtained by contact mainly in the second day after spray indicated that spores also can be protected from UV-radiation within the canopy suggesting that the period of time that TPB feed or exist on the canopy could be long enough to get infected. Overall, this investigation showed that the rapid inactivation of *B. bassiana* spores by solar radiation could be considered a major impediment to the success of the control of the TPB; nevertheless, the NI8 strain represents a valuable resource to be utilized within an IPM framework, and it will significantly contribute to reduction in chemical pesticide use.

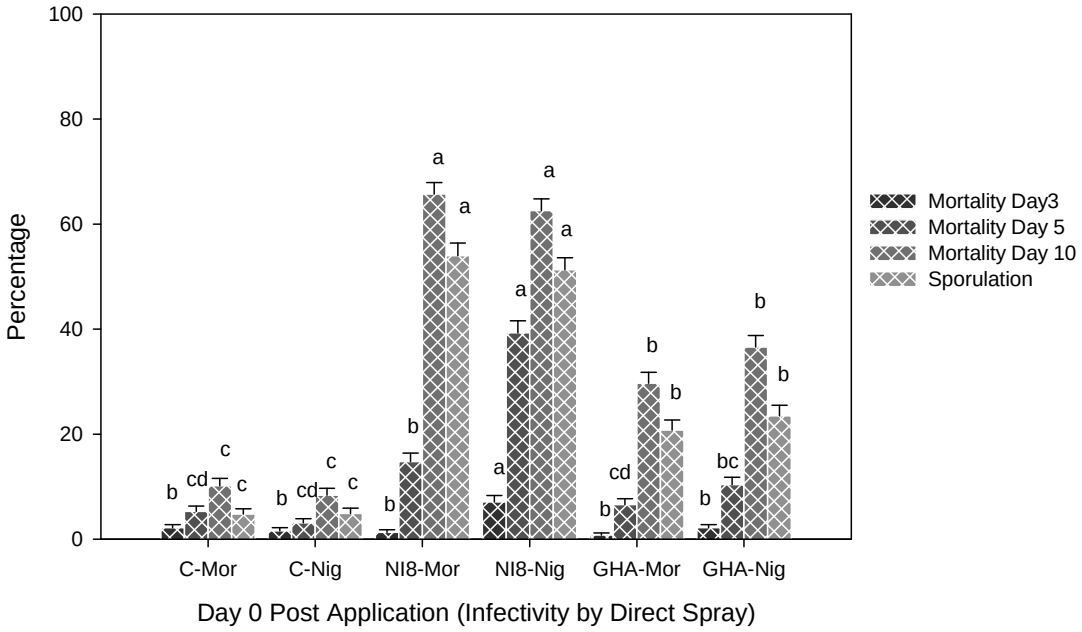

Figure1. Mortality of adults of *Lygus lineolaris* and sporulation percentage by directly spray during morning (Mor) and night (Nig) applications of *Beauveria bassiana* NI8 and GHA on cotton plants ($10x10^{12}$ spores/acre).

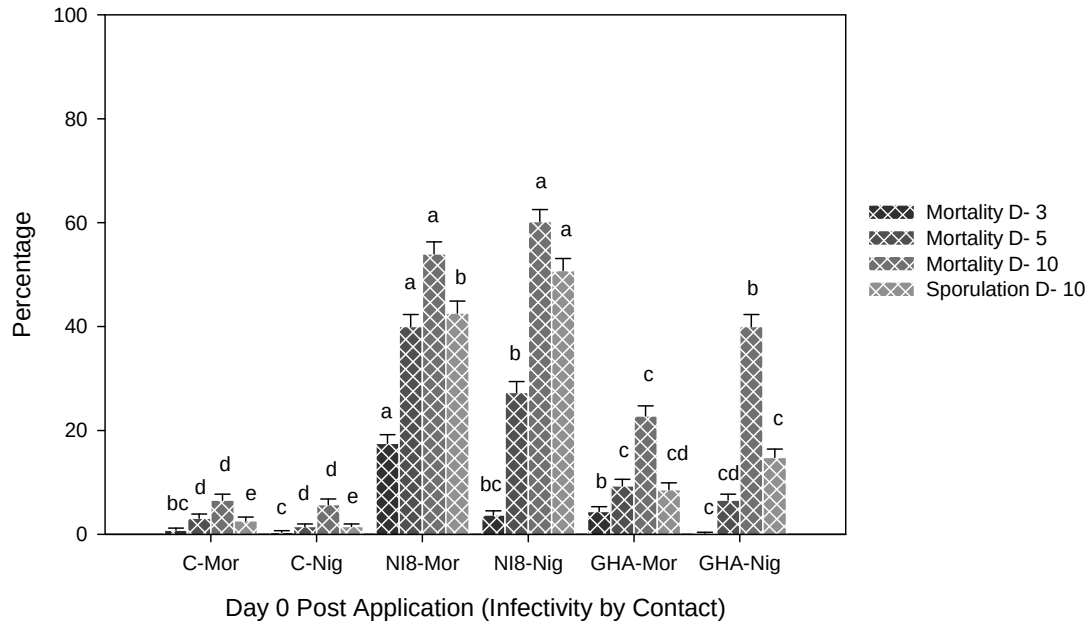

Figure2. Mortality of adults of *Lygus lineolaris* and sporulation percentage by contact on insects exposed 24 hours to sprayed cotton branches during morning (Mor) and night (Nig) applications with *Beauveria bassiana* NI8 and GHA ($10x10^{12}$ spores/acre).

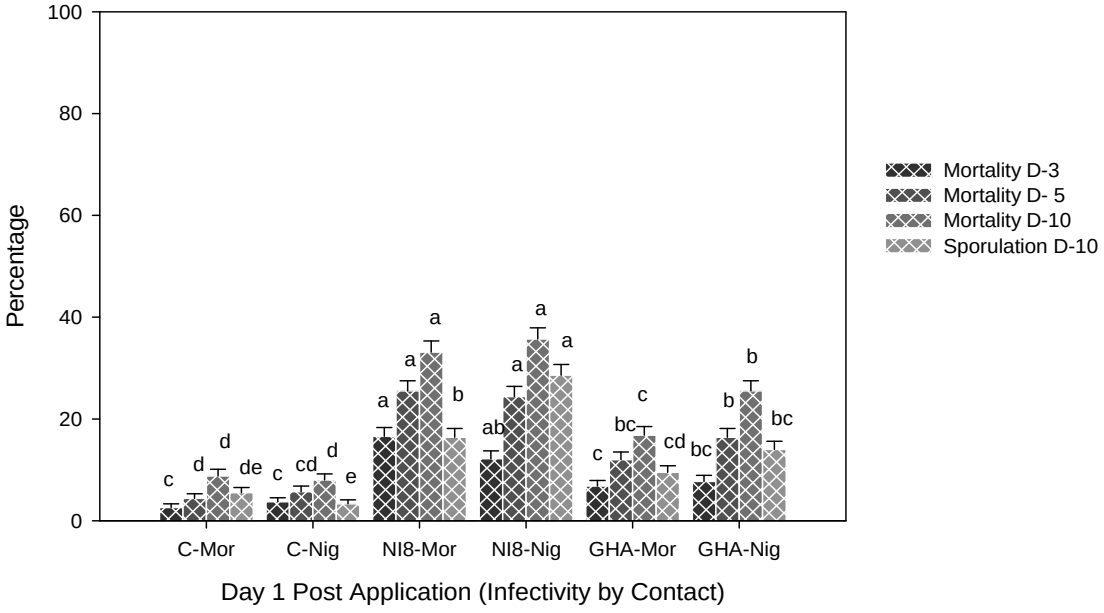

Figure3. Mortality of adults of *Lygus lineolaris* and sporulation percentage by contact on insects exposed 24 hours to sprayed cotton branches during morning (Mor) and night (Nig) applications with *Beauveria bassiana* NI8 and GHA ($10x10^{12}$ spores/acre).

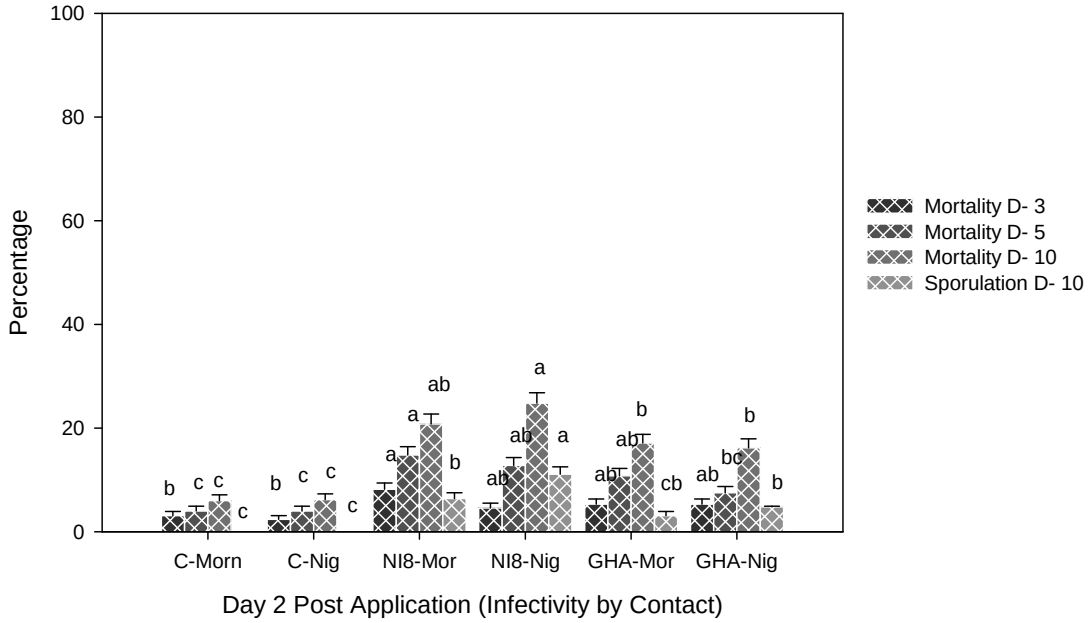

Figure4. Mortality of adults of *Lygus lineolaris* and sporulation percentage by contact on insects exposed 24 hours to sprayed cotton branches during morning (Mor) and night (Nig) applications with *Beauveria bassiana* NI8 and GHA ($10x10^{12}$ spores/acre).

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