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Mississippi Delta Native Strain of *Beauveria bassiana* for Control of Tarnished Plant Bug (*Lygus lineolaris*)

Report

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Abstract: Previous works reported that *Beauveria bassiana* (Balsamo) isolates from tarnished plant bug, TPB, *Lygus lineolaris* Palisot de Beauvois, (Hemiptera: Miridae) have shown a great potential to be used as a microbial control for TPB, particularly the NI8 strain, isolated from TPB from the Mississippi Delta. The Mississippi Delta native strain NI8 has been studied for more than ten years. Results from those studies demonstrated that NI8 strain has better characteristics of pathogenicity based on LC_{50} values, *in vitro* conidia production, temperature growth optima, tolerance to solar radiation, and production of beauvericin than the commercially available strain, GHA. The NI8 strain may offer an alternative to chemical pesticides protecting natural enemies without leading to secondary pests.

Keywords: Entomopathogenic fungi, Microbial pesticide, Tarnished plant bug, Delta strain NI8, *Beauveria bassiana*.

Introduction

Tarnished plant bug, TPB, *Lygus lineolaris* Palisot de Beauvois, (Hemiptera: Miridae) is an important insect pest of many crops in the United States. Direct economic damage results from feeding by nymphs and adults on reproductive parts of cotton plants. TPB is economically important in cotton through reductions of crop yields caused by flower bud abortion, death of plant terminals, and lint quality. In the United States, TPB infested more than 3 million hectares of cotton in 2006, resulting in a yield loss of more than 240,000 bales ($75 million based on a 218 kilogram (kg) bale and $1.43/kg) (Portilla et al. 2013). Across the midsouth states of Arkansas, Louisiana, and Mississippi during 1991-2005, TPB infested 77-99% of cotton acreage (Leonard and Cook, 2007).

Tarnished plant bug in cotton has been controlled for decades in the Mississippi Delta due to insecticidal treatments for lepidopterans pests. There are several factors that have contributed to the current status of TPB as the number one pest of cotton in this region necessitating the need for alternative control methods. These factors include reduction of insecticidal applications due to introduction of transgenic crops, success of boll weevil eradication program, and development of resistance to some commonly used insecticides. In 1993 TPB populations in the Mississippi Delta were found to be highly resistant to pyrethroid with multiple

resistances to some organophosphates and cyclodine insecticides (Snodgrass 1996, Hollingsworth et al. 1997, Snodgrass and Scott 2002, Snodgrass et al., 2009). Since then, alternative control measures for TPB in Mississippi have been studied including biological control (Smith and Nordlund 2000), sterile insect technique (Villavaso 2005), alternate host plant management (Snodgrass and Scott 2002, Snodgrass et al 2006, Lund et al. 2006a, Abel et al 2007), and microbial control agents (Snodgrass and Elzen 1994, Leland and Snodgrass, 2005; Leland 2005; Leland et al., 2005, McGuire et al., 2006, Lund et al. 2006b, Ugine 2012, Portilla et al. in press).

It is clear that complexity of the population dynamics in weedy hosts from season to season has made it unfeasible to target this insect with any single type of control. However, a culmination of experiences gained from the last 30 years of research is being applied to development of more expansive approaches to management of TPB in Mississippi by use of microbial biopesticides. Entomopathogenic fungi may have the greatest potential among microbial control agents for controlling sucking insect pests such as TPB because of the fungi's contact mode of action (Tanada and Kaya 1993). On the other hand, Leland (2005) mentioned that microbial biopesticides would be particularly well suited for TPB management because chemical insecticides are not labeled for TPB control on wild host plants and early season chemical application could increase resistance.

Beauveria bassiana as an alternative control of TPB in cotton

For more than 20 years, the entomopathogenic fungus *Beauveria bassiana* (Balsamo) Vuillemin (Hypocreales: Clavicipitaceae) has been proposed and investigated as an alternative method of control for TPB in cotton. Results of these investigations showed that *B. bassiana* was moderately effective in reducing TPB in cotton at a rate of 1.1 liter/ha (53.8 per cent and 20.2 per cent of nymphs and adult populations, respectively) (Snodgrass and Elzen 1994). Noma and Strickler (1999 and 2000) also reported low TPB adult mortality, but TPB nymphs were less vulnerable than adults according to Lund et al. (2006a). This fungus is extremely sensitive to high temperatures and solar radiation (Leland and Behle 2004, 2005). Spurgeon (2010) concluded that use of *B. bassiana* as a rescue treatment against *Lygus* in cotton may not be the most appropriate role for this pathogen. In 2002, an isolate of *B. bassiana* was found naturally infecting TPB in the Mississippi Delta. It was referred to as either NI8 strain or TPB 3 (Leland and Snodgrass, 2005). A number of different studies using this strain provided more encouraging results and advocated additional research for its use as an alternative TPB control measure. Leland (2005), Leland et al. (2005), McGuire et al. (2005, 2006) found the native delta strain was the most promising isolate for control of TPB in the Mississippi Delta based on LC_{50}, infection, and conidia production. Velez et al. (1997) and McGuire (2002) suggested that isolates obtained from the environment and host would be more effective in controlling the target pest. A brief review of published results on the native delta strain, NI8, and some implications for managing TPB in Mississippi are presented herein.

Beauveria bassiana isolates from TPB of Mississippi and factors influencing efficacy

Pathogenicity is the qualitative ability of a pathogen to cause disease It is determined by a variety of factors, including physiology of the host, physiology of the fungus, and the environment (Boucias et al. 1988 and 1991). Fungi have one of the widest host ranges among pathogens of arthropods. It is well recognized that *B. bassiana* contain a diverse assemblage of genotypes and probably include "species complexes" (Inglis et al. 2001). Selection of virulent genotypes is an important consideration for efficacy of microbial control of insects. Leland and Snodgrass (2005) conducted two surveys of natural *B. bassiana* infection levels in native TPB population from 20 counties in the delta hills, and mid-delta regions of Mississippi; one in 2003 and another in 2004. They found that the incidence of natural *B. bassiana* infection of TPB from wild host plants in this region was about 30 times lower than that previously reported for *L. hesperus* in the

San Joaquin Valley of California (0.3% versus 10%) (McGuire 2002). However, both species appear to be similarly susceptible to infection by *B. bassiana* (Leland and McGuire 2004).

Laboratory studies

The majority of studies evaluating the efficacy of an insecticide against TPB have been conducted using the commercial isolate of *B. bassiana* (ARSEF 6444) formulated as Mycotrol (Emeral Bioagriculture) (Brown et al. 1997, Noma and Strickler 1999 and 2000, Steinkraus 1996, Steinkraus and Tugwell 1997). One study evaluated efficacy of *B. bassiana* (ARSEF 3097) formulated as Naturalist (Snodgrass and Elzen 1994). Over the last ten years several investigations of *B. bassiana* isolated from *Lygus spp*. in the Mississippi Delta have been studied against *Lygus spp*. and compared to the current commercial strain GHA (ARSEF 128924) formulated as BotaniGard (Mycotech USA, Wraight et al. 2001) (Leland and Snodgrass 2005, Leland 2005, Leland et al. 2005, McGuire et al. 2005, Lund et al. 2006a and 2006b, Ugine 2011 and 2012, Portilla et al. 2013 and Portilla et al., in press).

Leland (2005) compared 19 *B. bassiana* isolates from Mississippi, a single *B. bassiana* isolate from TPB in Arkansas, and the commercial *B. bassiana* strain, GHA. Characteristics evaluated included pathogenicity to TPB adults, in vitro conidial production, tolerance to artificial sunlight, and germination at 35°C. Leland concluded that 11 strains of *B. bassiana* were significantly more pathogenic than the commercial strain based on LC_{50} values; with several having LC_{50} values more than 10 times lower that *B. bassiana* GHA. He also found that the conidia production from TPB isolates were similar or more prolific conidia producers than the GHA strain. Comparable LC_{50}s values were found by Portilla et al. (MP, unpublished data) among three strains from Mississippi (NI8, NI9, and GS1), three strains from California (MM1, MM2, and MM3), a strain from Arkansas, and the commercial strain, GHA. The NI8 strain had the lowest LC_{50} value among all strains and MM1 and GHA had the highest. All isolates were obtained from a collection at ARS-USDA-SIMRU (Stoneville MS) and were 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).

Other laboratory studies have shown high virulence of the NI8 strain. Leland et al. (2005) found that the Delta native strain, NI8, (Washington County) had higher in vitro conidia and beauvericin production than the commercial strain, GHA. Based on microsatellite markers, Leland et al. (2005) also found that the NI8 strain was more closely related to an isolate from TPB in Arkansas than to other TPB isolates from Washington county in Mississippi. Ugine (2011) evaluated effect of temperature on the NI8 strain concluding that *B. bassiana* caused a large reduction in total number of eggs laid at all temperatures. Portilla (MP, unpublished data) determined the effect of different temperatures on two strains of *B. bassiana* activity (NI8 and GHA) against TPB and temperature requirements for conidial germination for both *B. bassiana* strains. High mortality and sporulation of NI8 were obtained from 12°C to 30°C; low mortality and low sporulation were found at 10°C; and low mortality and no sporulation were obtained at 4 and 7°C for this strain. Significantly lower mortality and lower sporulation of GHA strain were found at all temperatures when compared with NI8; and low mortality and no sporulation were recorded at 10 °C or lower temperatures for the GHA strain.

Field studies

Field studies have demonstrated high pathogenicity of the NI8 strain against TBP. Portilla et al. (2013) evaluated two isolates of *B. bassiana* for pathogenicity and infectivity against TPB in the field: the commercial GHA strain and the NI8 strain. Thirty, 2 day old TPB adults from a laboratory colony were caged and placed on top and bottom parts of cotton plants in the field prior to spraying with *B. bassiana*. Sprayed insects were immediately collected from the cages and placed individually on *Lygus* solid diet and observed for ten days under laboratory conditions. Differences in mortality and sporulation on days 3, 5 and 10 were significant among concentrations for both isolates and placement positions. Estimates were determined by probit

analysis of concentration-mortality (top and bottom) and concentration-sporulation (top and bottom) relationships 10 days after spray based on LC_{50} values (lethal concentration) and LS_{50} values (lethal sporulation). *Beauveria bassiana* native NI8 strain with a LC_{50} of $6.5x10^{12}$ and LS_{50} of $1.7x10^{13}$ (spores/acre) was more infectious to TPB than the commercial GHA strain with a LC_{50} of $4.74x10^{14}$ and LS_{50} of $2.27x10^{15}$ (spores/acre). Overall, these results indicated that *B. bassiana* NI8 strain 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 approximately 73-fold lower than that of the commercial GHA strain. McGuire et al. (2006) demonstrated that NI8 also showed high virulence in the field to *Lygus hesperus*, Knight (Hemiptera: Miridae) when compared with strains from California and the commercial GHA strain. However, despite its high level of infection, no significant reduction of adult population occurred until 10-14 days after application.

Lund et al. (2006a and 2006b) compared *B. bassiana* strain NI8 and/or insect growth regulator Diamond (Novaluron) in midsouth cotton in Arkansas. They reported that there was no difference in average day to death in the *B. bassiana* or Diamond treatments singly or in combination, although, a more rapid death was observed in the combination of *B. bassiana* and Diamond. Snodgrass (2014, personal communication) indicated that sporulation percentage was higher in cotton plots sprayed with *B. bassiana* and Diamond than *B. bassiana* only treatment. Portilla, (Portilla et al., in press) found that fourth instar (97.5 ± SE 0.02), fifth instar (95.0 ± SE 0.03) and adults (95 ± SE 0.03) of TPB were more susceptible (infection %) than second (52.5 ± SE 0.07) and third instar (85.0± SE 0.05) to *B. bassiana*; while, second instar (100%), third instar (100%) and forth instar (97.5 ± SE 0.02) nymphs had higher growth inhibition (mortality %) than fifth instars (92.5 ± SE 0.04) after ten days of exposure to Novaluron. No effects in longevity (days) were observed in adults (21.57 ± SE 0.9) treated with Novaluron when compared with control insects exposed to water alone (20.47 ± SE 1.2), but both had a highly significant greater longevity than adults exposed to *B. bassiana* (5.2 ± SE 0.2).

Environmental factors

A variety of environmental factors have shown to have dramatic effects on the efficacy of entomopathogenic fungus *B. bassiana* against insect pests. However, one of the most important parameters affecting propagule persistence in epigeal habitats is deactivation by solar radiation (Inglis et al. 1995, 2001). Leland (2005) exposed several strains of *B. bassiana* including NI8 to artificial sunlight, concluding that all isolates were highly susceptible to negative effects of artificial sunlight, with only one isolate from Mississippi (NI8) germinating at 35°C. Leland et al. (2005) obtained faster conidia growth at 32°C in isolates from *Lygus spp*. than the growth obtained in GHA; however higher tolerances to artificial sunlight were observed on isolates from *L. hesperus* than on *L. lineolaris,* including NI8. Portilla et al. (MP, unpublished data) also found that significant differences among concentrations were observed in mortality and sporulation on TPB exposed to sprayed cotton branched for 24 hours. Mortality and sporulation drastically decreased from 2-fold by the next day to 18-fold by the third day after *B. bassiana* application. This reduction was observed primarily during August followed by July. Approximately 10 per cent of sporulation was found three days after *B. bassiana* application only in the highest concentration ($4x10^{13}$ spores/acre) in the month of June. In general, these results showed that TPB can be easily infected by contact mode of action. Survival of *B. bassiana* spores in the environment decreased with increasing temperatures (July and August) resulting in reduced efficacy from one day to another. Ultraviolet radiation from sunlight will continue to be the most detrimental environmental stress factor that highly affects use of this entomopathogenic fungus for control of TPB. However, because its ability to kill nearly 60 per cent of the adult population by contact alone, this microbial agent should still be considered to have a great potential for TPB control.

Conclusion

In general, the overview of different studies of *B. bassiana* NI8 strain suggested more control in TPB population than the current commercial GHA strain. However, it is unlikely that this

entomopathogenic fungus will ever supplant management of TPB by the use of chemical insecticides. Nevertheless, the NI8 strain represents a valuable resource when used within an IPM framework, and will significantly contribute to reduction in chemical pesticide use.

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