CONTROL OF TARNISHED PLANT BUG WITH *BEAUVERIA BASSIANA* AND INTERACTIONS WITH IMIDACLOPRID

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Abstract

The fungal entomopathogen, *Beauveria bassiana*, was isolated from a naturally infected tarnished plant bug, *Lygus lineolaris*, in Arkansas. In laboratory tests, this strain, ARSEF 3769, was highly infective to tarnished plant bug nymphs and adults. The LC_{50} for nymphs and adults was $9x10^4$ and $8.4x10^4$ spores per ml when mortality was recorded 7 days after treatment. In field tests, cotton plants treated with aqueous suspensions of *B. bassiana* at a rate of $5.8x10^7$ spores per ml resulted in 88.8% and 100% mortality (n=143) in exposed *L. lineolaris* adults at 5 and 7 days after treatment, respectively, compared with 7.4% and 11.5% in the controls (n=150). Field tests in 1995 with the commercial *B. bassiana* product, Mycotrol, and the insecticide imidacloprid, resulted in 97.9% mortality at 5 days after treatment in *L. lineolaris* adults when Mycotrol (280 g per ha) and Imidacloprid (50 g a.i. per ha) were applied together, compared with 9.5% mortality in the controls. The combination of Mycotrol and imidacloprid was significantly more effective than either material by itself. The *fungus B. bassiana* may useful for control of *L. lineolaris* in cotton.

Introduction

Tarnished plant bug (TPB), *Lygus lineolaris* (Palisot de Beauvois), is a serious cotton pest in the United States. Adult TPB are highly mobile and feed on a wide variety of plants. TPB reduces cotton yields by causing square shed, aborted terminals, and damaged anthers and bolls. TPB populations have recently shown resistance to insecticides. In addition, TPB may increase as a problem when Bt cotton results in reduced insecticide applications for worms.

Natural enemies, such as pathogens, predators and parasitoids could be useful in control of TPBs. Of the pathogens the fungi have the most potential because *L. lineolaris* has piercing sucking mouthparts and rarely becomes infected by bacteria, protozoa or viruses. Plant bugs were reported susceptible to *Beauveria bassiana* (Balsamo) Vuillemin by Bajan and Bilewicz- Pawinksa (1971) and Bidochka et al. (1993). However, natural infections of *L. lineolaris* have not been previously reported. In 1993 I collected a TPB in Arkansas naturally infected with *B. bassiana*. The fungus was isolated and deposited with the USDA fungal collection as ARSEF 3769.

The objectives of this study were to determine the infectivity of *B. bassiana* to TPB nymphs and adults, determine the time the fungus took to kill TPB, and test *B. bassiana* in the field.

Materials and Methods

Source of TPB

TPB were obtained for laboratory bioassays from a laboratory colony maintained on potatoes. TPB for field studies were collected by sweepnet from flowering plants prior to experiments. After collection, TPB were aspirated into buckets containing mustard flowers and held for 24 h. The bugs were then aspirated into individual cups and held in a cooler until placed in field cages.

Source of Beauveria and Imidacloprid

ARSEF 3769 was isolated from a TPB adult collected in alfalfa in Arkansas. The fungus was cultured on SDAY solid media for two weeks in the dark at 25°C, then spores were harvested and stored at 6°C until use. At time of use the spores were mixed with 0.01% Tween 80 in deionized water and used the same day for bioassays. Serial dilutions gave desired concentrations for experiments. Concentrations were determined using a hemacytometer and phase microscope. Spore viability was determined, at time of use, by plating the spore suspension on SDAY media. After 24 hours the number of germin-ated spores was counted using a phase microscope.

The recently registered *Beauveria* product, Mycotrol WP (Mycotech, Butte, Montana) was obtained for tests in 1995 as was the chemical, imidacloprid (Provado) (Bayer Agricultural Division, Atlanta, Georgia)

Laboratory Bioassays

TPB nymphs and adults were treated by placing them in a vial, adding the appropriate spore suspension or deionized water, and gently inverting the vial 5 times. The bugs were removed and placed in individual cups with a piece of fresh green bean. Five spore concentrations of ARSEF 3769 were tested: 10^4, 10^5, 10^6, 10^7, 10^8 spores per ml and a water control. Ten nymphs or adults were used per treatment, with 5 replicates of nymphs and 8 replicates of adults. Mortality was observed daily for 8 days. Results were analyzed by Polo-PC probit analysis.

Field Assays

In 1993 a field test in cotton was made at the Marianna Cotton Station in Arkansas. A ramdomized complete block design was used with 15 replicates per treatment, 10 adult TPB per replicate. Control plants were sprayed with water. Organdy sleeve cages, 0.6 by 0.3 m, were placed over the top five nodes and terminal of flowering cotton plants and tied around the stem. The spore suspension contained

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$5.8x10^7$ spores per ml and spore viability was 99.2% (n=1,566). Applications were made with a trigger type plant sprayer; 16 ml was applied per plant. Two treatments were compared: cotton plants sprayed before TPB were added ("Before" treatment) and cotton plants sprayed after TPB were added ("After" treatment). Five days after treatment surviving TPB were aspirated from the cages and placed individually in cups containing a piece of green bean. Dead TPB were daily.

A second field test was made 31 May 1995 at the Marianna Cotton Branch Station in a canola plot planted within a cotton field. A randomized complete block design was used; 5 replicates per treatment, 20 adult TPB per replicate. Bugs were caged on plants as in the 1993 field experiment. There were six treatments: deionized water control, Silwet L-77 Wetting Agent (0.04%) in deionized water, imidacloprid (50 g a.i. per ha), Mycotrol Low (280 g per ha), Mycotrol High (1.1 kg per ha), and imidacloprid (50 g a.i. per ha) plus Mycotrol (280 g per ha). Deionized water was used in all treatments and Silwet L-77 was added at 0.04% to all treatments except the water control and the imidacloprid treatment. Application was made with a CO_2 backpack sprayer at 20 gallons per acre, 34 psi and a flat fan Teejet 8002VS nozzle. Four days after treatment live TPB were aspirated from the cages and placed in individual diet cups with a 1 inch piece of fresh green bean. All dead TPB were counted and placed on moist filter paper to determine fungal infections. TPB mortality was recorded daily for 7 days after treatment. Dead TPB were placed in humid chambers to permit sporulation of infected insects and determine the number with frank mycoses. Data was analyzed by ANOVA and means separated by T-tests (LSD) (SAS Institute, 1987).

Results and Discussion

Laboratory Bioassays

Both adult and nymphal TPB were highly susceptible *to B. bassiana* ARSEF 3769. A dose response was observed. LC_{50} and LC_{90} values for nymphs and adults were calculated (Table 1). The data indicates that high concentrations are required to kill TPB quickly, however, low concentrations will eventually kill a large percentage of exposed TPB but may take 8 days. The LCs are based on the spore concentrations in the water used to immerse the bugs, not the number of spores that adhered to an individual bug. The actual numbers of spores necessary to infect and kill bugs are much lower than these numbers indicate.

The 1993 field test clearly indicates that aqueous spore suspensions of *B. bassiana* can be applied to cotton and infect and kill TPB adults under ambient field conditions. Temperatures during the test were hot (about 35°C during the day). Survival and recovery of TPB from control cages was excellent (148 bugs recovered out of a total of 150 placed in the control cages at start). In the control cages 92.6% of the bugs were still alive 5 days after treatment and 88.5% remained alive at 7 days after treatment. In contrast, only 11.2% (n=143) and 25.3% (n=142) of the bugs in the "Before" and "After" treatments were alive at 5 days after treatment and 100% of the bugs in both the "Before" and "After" treatments had died of frank mycoses by 7 days after treatment. This experiment showed that TPB could be killed by *B. bassiana* under field conditions either by directly spraying the bugs ("After" treatment) or when bugs contacted spores on plants previously sprayed ("Before" treatment).

In the 1995 field test, survival of bugs in the water (89.3%) and the Silwet (93.7%) controls was excellent at 4 days after treatment. Significant numbers of bugs were killed in the imidacloprid, imidacloprid plus Mycotrol, and Mycotrol Low and Mycotrol High rates (Table 2). By day 6 posttreatment mortality in the Mycotrol High treatment was significantly higher (83.9%) than imidacloprid by itself (67.3%), and mortality in the Mycotrol Low was similar (70.4%) to that in the imidacloprid treatment. Most interesting was the result that combining imidacloprid and Mycotrol Low resulted in significantly more mortality (97.9%) at 5 days posttreatment than any other treatment. Dead TPB held in moist conditions revealed that a high percentage of the bugs in the Mycotrol treatments and imidacloprid plus Mycotrol treatments died of frank mycoses caused by *B. bassiana* (Table 3).

These results indicate that *Beauveria bassiana* by itself could be an effective control agent for TPB in cotton if time is not a major factor. There may be situations, such as in alternate or trap crops, that are breeding areas for TPB, or in wind strips, conservation areas, and weedy field borders, where killing the TPB in 6-7 days is satisfactory. It is also apparent from the data that combining imidacloprid with *Beauveria bassiana* significantly increased the mortality in exposed bugs above that of either material alone. The reasons for this are unclear. Evidence from research on termites (Boucias et al. 1995) indicates imidacloprid altered that grooming and other social of the termites, increasing their susceptibility to fungal pathogens. Such a mechanism is unlikely in the case of non-social insects such as TPB. If the economics are favorable, it appears that a mixture of Mycotrol and imidacloprid will provide better control of TPB than imidacloprid alone.

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References Cited

Bajan, C. and T. Bilewicz-Pawinska. 1971. Preliminary studies on the role of *Beauveria bassiana* (Bals.) Vuill. in reduction of *Lygus rugulipennis* Popp. Ekologia Polska 19: 35-46.

Bidochka, M. J., G. S. Miranpuri, and G. G. Khachatourians. 1993. Pathogenicity of *Beauveria bassiana* (Balsamo) Vuillemin toward lygus bug (Hem., Miridae). J. Appl. Ent. 115: 313-317.

Boucias, D. G., C. Stokes, G. Storey, and J. Pendland. 1995. Effect of imidacloprid on both the termite, *Reticulotermes flavipes* and its interaction with insect pathogens. Pfanzenshutz-Natrichten Bayer (submitted).

SAS Institute, Inc. 1987. "SAS system for elementary statistical analysis." SAS Institute, Inc. Cary, NC.

Table 1. LC_{50} and LC_{90} values for *Lygus lineolaris* nymphs and adults immersed in *Beauveria bassiana* suspensions.

Day Post	LC_{50}		LC_{90}	
Treatment	nymph	adult	nymph	adult
4	$1.5x10^8$	-	$9.0x10^{11}$	-
5	$2.2x10^6$	$3.7x10^6$	$8.2x10^8$	$4.0x10^8$
6	$2.0x10^5$	-	$3.2x10^7$	-
7	$9.0x10^4$	$8.4x10^4$	$1.4x10^7$	$1.2x10^7$
8	$2.0x10^4$	-	$2.5x10^6$	-

5th instar nymphs were used and the bugs were immersed in a suspension of spores.

Table 2. Mortality in *Lygus lineolaris* (tarnished plant bug) in a 1995 field test in Arkansas.

	Mean Percentage Mortality Days Posttreatment			
Treatment	4	5	6	7
Imidacloprid + Mycotrol Low	86.3 a	97.9 a	98.9 a	98.9 a
Imidacloprid	63.5 a	67.3 b	67.3 b	75.0 ab
Mycotrol high	37.7 b	61.3 b	83.9 ab	91.5 a
Mycotrol low	27.6 bc	52.0 b	70.4 b	84.7 ab
Silwet Control	6.7 c	7.6 c	9.5 d	18.1 c
Water control	10.7 c	13.6 c	19.4 cd	27.2 c

Means with the same letter in a column are not significantly different. T-tests (LSD), Alpha=0.05 (SAS Institute, 1987)

Table 3. Percentage of *L. lineolaris* cadavers in 31 May 1995 field test that produced frank *B. bassiana* mycoses (i.e. sporulated).

Treatment	% Cadavers producing frank mycoses
Imidacloprid + Mycotrol low	79.8
Imidacloprid	0
Mycotrol High	87.5
Mycotrol Low	94.3
Silwet Control	0
Water Control	0