Beauveria bassiana (Deuteromycotina: Moniliales) Effects on *Lygus lineolaris* (Hemiptera: Miridae)[1]

D. C. Steinkraus and N. P. Tugwell

321 AGRI
Department of Entomology
University of Arkansas, Fayetteville, AR 72701 U.S.A.

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ABSTRACT The fungal entomopathogen, *Beauveria bassiana* (Balsamo) Vuillemin, was isolated from a naturally-infected tarnished plant bug, *Lygus lineolaris* (Palisot de Beauvois), in Arkansas. This is the first report of a tarnished plant bug naturally infected with *B. bassiana.* In laboratory tests this isolate (ARSEF 3769) was highly infective to *L. lineolaris* nymphs and adults. The LC_{50} for nymphs and adults was 9×10^4 and 8.4×10^4 conidia per ml deionized water containing 0.01% Tween 80, respectively, when mortality was recorded 7 days after treatment. In a 1993 field test, cotton plants treated with ARSEF 3769 conidia at a rate of 5.8×10^7 conidia per ml deionized water containing 0.01% Tween 80 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 11.4% mortality in the controls (n = 150). Persistence tests on field cotton showed that *B. bassiana* conidia could infect adult *L. lineolaris* for up to 4 days under ambient environmental conditions. A 1995 field test with the commercial *B. bassiana* product, Mycotrol WP, and the insecticide imidacloprid (Provado formulation) on *L. lineolaris* adults caged on canola 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 67.3% in the imidacloprid alone (50 g a.i. per ha), 52% in the Mycotrol alone (280 g per ha), and 7.6% and 13.6% mortality in the controls. The combination of Mycotrol and imidacloprid was significantly more effective than either material alone. These studies show that the fungus *B. bassiana* may be useful for control of *L. lineolaris* in cotton and other crops.

KEY WORDS *Lygus lineolaris,* Miridae, *Beauveria bassiana*, imidacloprid, cotton

Tarnished plant bug, *Lygus lineolaris* (Palisot de Beauvois), is a serious pest in the United States. Adults are highly mobile and feed on a wide variety of plants (Snodgrass et al. 1984, Young 1986, Scott 1987). This insect reduces cotton yields by causing square shed, aborted terminals, and damaged anthers and bolls (Tugwell et al. 1976, Hanny et al. 1977). Recently due to resistance, efficacy of insecticides for control of this insect has declined resulting in control failures (Cleveland 1985, Snodgrass and Scott 1988, Hollingsworth et al. 1997, Pankey et

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al. 1996). Introduction of genetically-engineered cotton plants expressing *Bacillus thuringiensis* Berliner delta-endotoxins that kill Lepidoptera larvae (Barton et al. 1987) in conjunction with boll weevil, *Anthonomus grandis* Boheman, eradication programs in the southern U.S. may result in increased plant bug problems. This is because fewer insecticide applications may be made for Lepidoptera or boll weevils, applications that had incidentally also killed plant bugs.

Natural enemies could be useful in *L. lineolaris* control. Much work has been done on the hymenopteran and dipteran parasitoids of Miridae (Clancy and Pierce 1966, Hedlund and Graham 1987, Day et al. 1990, Day 1995). Generally, natural parasitization of *L. lineolaris* has been relatively low in the United States (8 to 13%). Introduction of braconid parasites from Europe led to increased parasitism of *L. lineolaris* in New Jersey (Day et al. 1990). In contrast to studies on hymenopteran or dipteran parasitoids of *Lygus* spp., few studies have been made on the natural or applied control of *Lygus* spp. with entomopathogens.

Of the pathogens, entomopathogenic fungi have the most potential for control of Homopterans (Carruthers and Soper 1987, Tanada and Kaya 1993) because the portal of entry for entomopathogenic fungi is generally directly through the host integument. Several mirid species have been reported to be naturally susceptible to two species of Entomophthorales. *Lygus communis* var. *novascotiensis* Knight, *Plagiognathus* sp. (Dustan 1924), *Irbisia solani* (Heidemann) (Hall 1959), *Adelphocoris lineolatus* (Goeze) (Wheeler 1972), and *Notostira elongata* Geoffrey (Keller 1981, Ben-Ze'ev et al. 1985) have been found infected by *Entomophthora erupta* (Dustan) Hall. *Notostira elongata* (Ben-Ze'ev et al. 1985) has been found infected by *E. helvetica* Ben-Ze'ev, Keller and Ewen.

Lygus rugilipennis Poppius was reported to be infected naturally by the deuteromycete *Beauveria bassiana* (Balsamo) Vuillemin by Bajan and Bilewicz-Pawinka (1971). Riba et al. (1986) reported epizootics caused by *B. bassiana* in the mirids *L. rugulipennis* and *A. lineolatus*. Bidochka et al. (1993) found that a mixed population of *L. borealis* (Kelton), *L. desertinus* Knight, and *L. lineolaris* was susceptible in the laboratory to conidia of *B. bassiana* GK 2016 (Bioinsecticide Research Laboratory, University of Saskatchewan), but they made no attempt to determine susceptibility of individual species or quantify dosage. In addition, control of *L. lineolaris* by the commercially-produced *B. bassiana* product, Naturalis-L (Troy Biosciences, Phoenix, AZ) was reported by Snodgrass and Elzen (1994). Natural infections of *L. lineolaris* by *B. bassiana* have not been reported previously.

Imidacloprid is a new class of insecticide with activity against sucking insects such as aphids, whiteflies, and *Lygus* bugs (Elbert et al. 1990, Teague and Tugwell 1996). Recent research has shown that imidacloprid enhances the pathogenicity of fungal pathogens to first-instar larvae of the citrus root weevil, *Diaprepes abbreviatus* (L.) (E. D. Quintela and C. W. McCoy, University of FL, Lake Alfred, FL, unpublished data) and termites (Boucias et al. 1996). Teague and Tugwell (1996) found reduced feeding in *L. lineolaris* fed sublethal doses of imidacloprid.

The objectives of this study were: (1) to isolate *B. bassiana* from a naturally-infected *L. lineolaris* adult, (2) to determine the infectivity of this natural

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isolate to tarnish plant bug nymphs and adults, (3) to determine the time the fungus took to kill tarnished plant bugs, (4) to determine how long *B. bassiana* on cotton in the field remained infective to tarnished plant bugs, (5) to test a natural *B. bassiana* isolate and the commercially-produced *B. bassiana* product, Mycotrol WP, in the field for tarnished plant bug mortality, and (6) to determine whether combinations of imidacloprid and *B. bassiana* were more effective than the separate materials.

Materials and Methods

Sources of tarnished plant bugs. Tarnished plant bugs were obtained for laboratory bioassays from a laboratory colony maintained on sprouting potatoes (Slaymaker and Tugwell 1982). Wild *L. lineolaris* for field studies were collected prior to experiments by sweepnet from flowering mustard [*Brassica juncea* (L.) Cosson], canola (*Brassica napus* L.), or various fleabanes (*Erigeron* spp.). After collection, tarnished plant bugs were aspirated from the sweepnets and placed in 13.2-liter organdy-covered plastic pails containing flowering terminals of the host plants. Twelve hours later, actively moving *L. lineolaris* were aspirated from the pails into 50-ml plastic vials and held in a cool ice chest until placed in field cages.

Sources of *Beauveria* and imidacloprid. In 1992, we collected a wild adult tarnished plant bug in Arkansas naturally infected with *B. bassiana*. The specimen was collected by sweep net from alfalfa (*Medicago sativa* L.) in Little River Co., AR, on 17 June 1992. The fungus was isolated and deposited in the USDA-ARS Entomopathogenic Fungal Culture Collection, Ithaca, NY, accession number ARSEF 3769.

Before use in tests with ARSEF 3769, the fungus was cultured on Sabouraud's dextrose agar (Difco, Detroit, MI) containing 1% Bacto yeast extract (Difco, Detroit, MI) solid media (SDAY) for 2 wks in the dark at 25°C. Conidia were then harvested and stored at 6°C until used. At time of use in all experiments, except the 1995 field test, the conidia were mixed with 0.01% Tween 80 (polyoxyethylene sorbitan monooleate) (Sigma, St. Louis, MO) in deionized water and used the same day for bioassays. Serial dilutions gave desired concentrations for experiments. Concentrations were determined by counts of the average number of conidia per ml in the stock suspension (two samples from a stock suspension, five counts per sample) using a hemacytometer and phase microscope (Cantwell 1970). Spore viability was determined at time of use by plating 0.1 ml of the spore suspension diluted to 1×10^7 conidia per ml on each of two 9-cm Petri dishes containing SDAY media then holding the dishes for 24 h at 25°C in the dark (Clerk and Madelin 1965, Steinkraus et al. 1991). A drop of lactophenol-acid fuchsin stain and a coverslip were placed on each Petri dish. Then the number of germinated and ungerminated conidia in five areas of each coverslip at 400x under a phase microscope were counted and the average germination rate was determined. ARSEF 3769 was used in all tests except the 1995 field test.

The recently-registered *B. bassiana* product, Mycotrol WP (Mycotech, Butte, MT) was used in the 1995 field test, as was Provado, a commercial formulation of imidacloprid (Bayer Agricultural Division, Atlanta, GA).

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Laboratory bioassays. ARSEF 3769 was used in laboratory bioassays to determine the LC_{50} and LC_{90} for fifth-instar nymphs and adults of *L. lineolaris*. Ten nymphs or adults were placed in each 40-ml screw cap vial. Then 10 ml of the appropriate spore suspension concentration was added, and the vial was inverted gently five times. Insects were removed from the spore suspension by pouring the vial contents through 1-mm mesh nylon tulle netting. Then they were collected with a fine tip brush and transferred individually to 30-ml plastic cups containing a 2.5-cm piece of fresh green bean. After the insects had dried, the cups were capped, labeled, and held at 25°C (12:12 LD). Fresh green bean pieces were added as necessary. Six spore concentrations of ARSEF 3769 were tested: 0, 10^4, 10^5, 10^6, 10^7, 10^8 conidia per ml deionized water containing 0.01% Tween 80. Ten nymphs or adults were used per treatment, with five replicates of nymphs and eight replicates of adults. Mortality was recorded daily after treatment for 8 days (nymphs) or 5 and 7 days (adults). Results were analyzed by Polo-PC probit analysis (LeOra Software, Berkeley, CA).

1993 field assay on cotton. On 15 July 1993, a field test in cotton was conducted at the Cotton Branch Station, Lee Co., AR. The 1-ha cotton field was planted with 'Deltapine 51' (Delta and Pine Land, Scott, MS) on 17 May. Flowering mustard was planted along the south side to attract tarnished plant bugs for collection and use in the test. Nylon tulle sleeve cages (1-mm mesh), 0.6 by 0.3 m, were placed over the top five nodes and terminal of flowering cotton plants and attached at the stem. Cages were separated by 5 m down row and 3 m across rows. Adult *L. lineolaris* were collected from mustard the day before the experiment as described above. A spore suspension containing 5.8 $x10^7$ conidia per ml (ARSEF 3769) was prepared. Application of 16.4 ml per plant was made with a trigger-pump hand sprayer at 1800 to 1900 h. The three treatments were: (1) indirect (plants sprayed before insects were added to cages), (2) direct (plants sprayed after insects were added to the cages), and (3) control (insects sprayed on the plants with deionized water plus 0.01% Tween 80). A randomized complete block design was used with 15 replicates per treatment, 10 insects per replicate. Five days after treatment live and dead insects were aspirated from the cages. Live insects were placed individually in 30-ml cups containing a 2.5-cm piece of fresh green bean. Mortality was recorded daily starting on day 5 after treatment, and the proportion of dead insects per replicate was determined. The proportions were transformed by square root/arcsine, then ANOVAs run on the 5 and 7 day data. Means were separated by the Tukey-Kramer test (Sall and Lehman 1996). Temperatures and rainfall during the test were obtained from the Cotton Branch Station weather station.

Persistence of *Beauveria* on cotton plants. Tests were conducted in the field on 'DPL 50' cotton planted on 19 May 1994 in Washington Co., AR, to determine the persistence of *B. bassiana* infective conidia on cotton plants under ambient environmental conditions. ARSEF 3769 was produced and handled as in the laboratory bioassays. Cages and insect handling were the same as in the 1993 field test described above. The test was conducted twice (25 July and 15 September 1994). The rate for both tests was $5x10^7$ conidia per ml, with 15 ml applied to each plant with a trigger-pump hand sprayer at 1330 hours. A randomized complete block design was used in each test, with 10 adult *L. lineolaris* per cage, 10 replicates per treatment in the first test, and 15 insects per

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cage, five replicates per treatment in the second test. The four treatments were: (1) control (insects on plants in cages sprayed with water and 0.01% Tween 80), (2) direct (insects on plants in cages sprayed directly with the *B. bassiana* suspension), and (3 and 4) 24 h and 96 h persistence (plants were sprayed with conidial suspension then 24 or 96 h later insects were placed on the caged plants). Three days after placement in their respective cages, live and dead insects were aspirated from the cages. Live insects were placed in individual 30-ml plastic cups containing a 2.5-cm piece of fresh green bean and covered with a paper lid. Dead insects were placed in sporulation chambers [9-cm Petri dish with a moistened piece of filter paper, sealed with Parafilm® (American National Can, Greenwich, CT)]. These chambers provided suitable conditions for *B. bassiana* sporulation on infected *L. lineolaris*. Tarnished plant bugs in sporulation chambers were checked daily for sporulation of *B. bassiana* to provide positive confirmation that insects were killed by the pathogen. Mean proportion mortality at 7 days after exposure to *B. bassiana* conidia on the plants was transformed by square root/arcsine and analyzed by ANOVA. Means were separated using the Tukey-Kramer test (Sall and Lehman 1996). Temperature and rainfall data for the period of each test were obtained from the weather station at the Agricultural Experiment Station Farm in Washington Co., AR.

1995 field experiment. A field test was initiated 31 May 1995 at the Cotton Branch Station, Lee Co., AR, in a flowering canola plot planted within a cotton field. The canola was planted on 1 November 1994, and 'Deltapine 51' cotton was planted 15 May 1995. A randomized complete block design was used, five replicates per treatment, 20 adult tarnished plant bugs per replicate. Adult *L. lineolaris* were caged on canola plants as in the 1993 field cotton experiment. Canola plants were used because the cotton was too small to use at this date when *L. lineolaris* were abundant on the flowering canola. Deionized water was used in all treatments, and Silwet L-77 surfactant (Helena Chemical, Memphis, TN) was added at 0.04% to all treatments except the water control and the imidacloprid treatment. Germination tests were not performed with the Mycotrol product. There were six treatments: (1) water control, (2) Silwet L-77 (0.04%) in water, (3) imidacloprid (50 g a.i. per ha), (4) Mycotrol low (280 g per ha), (5) Mycotrol high (1.1 kg per ha), and (6) imidacloprid (50 g a.i. per ha) plus Mycotrol low (280 g per ha). Application was made with a CO_2 backpack sprayer at 187 liters per ha, 2.39 kg/cm^2 (34 psi), and a flat fan Teejet 8002VS nozzle. Four days after treatment live and dead insects were aspirated from the cages. Live insects were placed in individual 30-ml plastic cups containing a 2.5-cm piece of fresh green bean. Mortality was recorded daily between 4 and 7 days after treatment. Each day, dead insects were placed into sporulation chambers to permit sporulation of infected insects and determine the number with mycoses. Temperature and rainfall during the test were obtained from the Cotton Branch Station weather station. Mean proportions of dead tarnished plant bugs by day were transformed by square root/arcsin and analyzed by ANOVA. Means were separated by *t*-tests (LSD) (SAS Institute 1987).

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Results and Discussion

Laboratory bioassays. Tarnished plant bug nymphs and adults were highly susceptible to *B. bassiana* ARSEF 3769 in the laboratory (Table 1). The germination rate of conidia used in the nymph tests was 96.5%, and that for the adult tests was 98.3%. The LC_{50} and LC_{90} values for nymphs and adults varied depending on the length of time post-inoculation. At 5 days after treatment the LC_{50} for nymphs was 2.2×10^6 conidia per ml compared to 2.2×10^4 conidia per ml at 8 days. The adult LC_{50} at 5 days was 3.7×10^6 conidia per ml.

Unlike many chemical insecticides, most entomopathogenic fungi take several days to kill an infected host. Bidochka et al. (1993) found that the LT_{50} for a mixed population of *L. borealis, L. desertinus,* and *L. lineolaris* treated in the laboratory with *B. bassiana* was 4.9 days, and 90% mortality was achieved by 7 days after the insects had walked over a sporulating, *in vitro* culture of *B. bassiana* (dosage was not quantified). Similar results were obtained by Bajan and Bilewicz-Pawinska (1971) when they sprayed nymphs or adults of *L. rugilipennis* with aqueous suspensions of *B. bassiana* at rates of 5×10^5 or 2×10^6 conidia per ml. They found no difference in susceptibility of nymphs or adults, and at the high rate, 100% of treated insects died by 5 days, but it took 10 days to kill 100% of the treated insects at the lower rate. Interestingly, Bajan and Bilewicz-Pawinska (1971) stated that *L. rugilipennis* was susceptible *per os* as well as through the integument and that this might permit infections to occur under conditions of low relative humidity in the field. Our data indicate that while relatively high concentrations of *B. bassiana* conidia were required to kill insects within 5 days, low concentrations killed a high percentage of insects within 8 days. Our laboratory-derived LCs are based on the spore concentrations in the water used to immerse the insects, not on the number of conidia that actually adhered to and infected individuals. The numbers of conidia necessary to infect and kill are undoubtedly much lower than the LCs indicate.

1993 field assay on cotton. Spore germination was 99.2% in this test (n = 1,566). Temperatures during the test were relatively hot, with daily high air temperatures ranging from 34.4 to 37.7°C (mean = 35.7°C, SE = 0.55). Daily low air temperatures ranged from 22.2 to 24.4°C (mean = 23.2°C, SE = 0.33). Rainfall during the test was 0.38 cm on 15 July, the day the test was initiated, and there was an additional 2.97 cm rain on 20 July, the day the tarnished plant bugs were removed from the cages.

The 1993 field test shows that aqueous spore suspensions of *B. bassiana* can be applied to cotton and infect and kill tarnished plant bug adults under ambient field conditions (Table 2). The nylon tulle cages have an open weave and large mesh so that conditions of temperature, light, and relative humidity were likely comparable to uncaged conditions. Significantly more insects survived in the control cages than in either *B. bassiana* treatment 5 and 7 days after treatment (Table 2). In the control cages, 92.6% of the insects were alive 5 days after treatment, and 88.6% (n = 148) were alive at 7 days after treatment. In contrast, only 11.2% (n = 143) and 24.9% (n = 142) of the insects in the direct and indirect treatments were alive 5 days after treatment, and 100% of the insects in both the *B. bassiana* treatments were dead by 7 days. Mortality in

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Table 1. LC_{50} and LC_{90} values for *Lygus lineolaris* nymphs and adults immersed in suspensions of *B. bassiana* ARSEF 3769 conidia.

Day after treatment	LC_{50}		LC_{90}		
	dose*	95% fiducial limits	dose*	95% fiducial limits	slope
		5th instar nymphs			
4	$1.5x10^8$	–	$9.0x10^{11}$	–	0.339
5	$2.2x10^6$	($6.8x10^4$ - $3.2x10^7$)	$8.2x10^8$	($4.8x10^7$)	0.499
6	$2.0x10^5$	($9.7x10^3$ - $1.1x10^6$)	$3.2x10^7$	($4.7x10^6$ - $4.0x10^9$)	0.584
7	$9.0x10^4$	($5.8x10^3$ - $4.3x10^5$)	$1.4x10^7$	($2.6x10^6$ - $4.4x10^8$)	0.585
8	$2.2x10^4$	($3.0x10^2$ - $1.2x10^5$)	$2.5x10^6$	($4.6x10^5$ - $1.4x10^8$)	0.623
		adults			
5	$3.7x10^6$	($5.8x10^5$ - $1.7x10^7$)	$4.1x10^8$	($6.3x10^7$ - $5.0x10^{10}$)	0.629
7	$8.4x10^4$	($1.2x10^4$ - $2.9x10^5$)	$1.2x10^7$	($3.0x10^6$ - $1.4x10^8$)	0.594

* Dose is expressed as number of viable conidia of *B. bassiana* per ml of 0.01% Tween 80 in deionized water.

Table 2. Mortality in *L. lineolaris* adults treated with conidia of *B. bassiana* ARSEF 3769 in a 1993 Arkansas cotton field test.

			Mean (SE) percentage mortality Days Posttreatment	
Treatment	# replicates	# TPB	5	7
Control	15	148	7.4 (2.9) a	11.4 (3.1) a
Direct	15	143	88.8 (2.5) b	100.0 (0.0) b
Indirect	15	142	75.1 (5.8) b	100.0 (0.0) b

Means with the same lower case letter within a column are not significantly different, Tukey-Kramer, $P = 0.05$, JMP (Sall and Lehman 1996). Anovas were run on the arcsine/square root of the proportion of dead insects per replicate on each date: for day 5, df = 2, F = 79.6, $P < 0.0001$; for day 7, df = 2, $F = 480.9$, $P < 0.0001$.

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the direct treatment was not statistically higher than that in the indirect, suggesting that in some unknown manner *L. lineolaris* efficiently contacted conidia on plants and did not need to be directly hit by spray droplets containing conidia. Of the few *L. lineolaris* remaining alive in the cages from the *B. bassiana* treatments on day 5 after treatment, many were moribund. This suggests that, while some insects survived 4 to 5 days after treatment, they were infected and may have been incapable of feeding on cotton squares. The timing and the extent to which tarnished plant bug feeding is curtailed by *B. bassiana* infection is a research question that needs to be addressed. It is also possible that infected *L. lineolaris* might be predated on more readily than healthy insects.

Many dead *L. lineolaris* removed on day 5 after treatment from *B. bassiana* treatments, then placed into moist sporulation chambers, resulted in profuse sporulation of the fungus. This was surprising because these infected insects had been dried by hot field temperatures (up to 37.7°C) and sunlight. This demonstrated that *B. bassiana* could survive within a dead infected host under hot, dry conditions and still sporulate. This suggests *B. bassiana* may be able to recycle in agroecosystems if infected insects end up in moist sites on the plant or soil. This experiment demonstrated that *B. bassiana* could kill *L. lineolaris* under relatively hot, dry, sunny field conditions on cotton.

Persistence of *Beauveria* on cotton plants. *Beauveria bassiana* germination for the 25 July test was 96.8% (n = 525) and was 96.1% (n = 412) for the 15 September test. Conidia on cotton plants remained infective for at least 96 hours (Table 3). However, there was a loss of infectivity over time. Mortality in directly sprayed *L. lineolaris* adults was 98.5% and 100% in the 25 July and 15 September tests, respectively, significantly higher than in treatments in which the conidia weathered for 24 hours (58.3% in the 25 July test and 43.3% in the 15 September test) or 96 hours (42.7% in the 15 September test) ($F = 71.2$; df = 2; $P < 0.0001$, 25 July test; $F = 81.5$; df = 3; $P < 0.0001$, 15 September test). Sporulation of *B. bassiana* on tarnished plant bug cadavers from fungal-treated plants approached 90% in directly treated insects (Table 3) but was inconsistent in the other treatments for unknown reasons. The data indicate that *B. bassiana* conidia can survive and infect *L. lineolaris* for 24 to 96 hours on cotton plants. Air temperatures ranged from highs of 23.3 to 32.2°C (mean = 27.4°C, SE = 1.6), and lows of 13.3 to 20°C (mean = 16.1°C, SE = 1.6) during 25 to 29 July. The respective temperatures for the period 15-22 September were 26.1 to 31.7°C (mean = 28.6°C, SE = 0.6) and 10.6 to 21.1°C (mean = 13.8°C, SE = 1.5). Rainfall during the two tests was 1.4, 0.9, 0.2 cm on 25, 27, and 28 July, respectively, and 0.7 cm on 22 September.

1995 field experiment. In the 1995 field test, insect survival in the water (89.3%) and the Silwet (93.7%) controls was excellent at 4 days after treatment (Table 4). Significantly more tarnished plant bugs were killed in the imidacloprid, imidacloprid plus Mycotrol low, Mycotrol low, and Mycotrol high rates than in either control (Table 4). Six days after treatment, mortality in the Mycotrol high treatment was higher (83.9%) than imidacloprid alone (67.3%), and similar in the Mycotrol low treatment (70.4%). Combining imidacloprid and Mycotrol low resulted in significantly higher mortality (97.9%) at five and six days posttreatment than with imidacloprid alone (Table 4). Dead insects held in sporulation chambers showed that many *L. lineolaris* in the Mycotrol treatments and imidacloprid plus Mycotrol treatment died of mycoses (Table 4).

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Table 3. Persistence *B. bassiana* conidia on cotton plants under field conditions in 1994 as shown by infectivity to *L. lineolaris* adults.

Treatment Date	Trt	# reps	# bugs	mean (SE) % dead at 7 d	% cadavers sporulating (n)
25 July	control	10	99	25.0 (5.4) a	0.0 (25)
	direct	10	115	98.5 (1.0) b	88.5 (113)
	24 h	10	97	58.3 (5.8) c	84.2 (57)
15 September	control	5	74	19.1 (5.3) a	0.0 (14)
	direct	5	74	100.0 (0.0) b	87.8 (74)
	24 h	5	74	43.3 (3.6) c	6.3 (32)
	96 h	5	75	42.7 (7.5) c	12.5 (32)

Means within a date within a column followed by the same lower case letter are not significantly different (Tukey-Kramer at a 0.05 probability level).

Table 4. Mortality and percentage mycosis of *L. lineolaris* adults on canola in a cotton field in a 1995 Arkansas field test.

	Mean percentage mortality Days posttreatment				
Treatment	4	5	6	7	% cadavers with mycosis
Imidacloprid+ Mycotrol low	86.3 a	97.9 a	98.9 a	98.9 a	79.8
Imidacloprid	63.5 a	67.3 b	67.3 b	75.0 ab	0
Mycotrol high	37.7 b	61.3 b	83.9 ab	91.5 a	87.5
Mycotrol low	27.6 bc	52.0 b	70.4 b	84.7 ab	94.3
Silwet control	6.7 c	7.6 c	9.5 d	18.1 c	0
Water control	10.7 c	13.6 c	19.4 cd	27.2 c	0

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

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Air temperatures during the test were moderate with daily highs ranging from 21.7 to 31.7°C (mean = 26.4°C, SE = 2.2), and lows ranging from 17.2 to 18.3°C (mean = 17.9°C, SE = 0.3), and there was 1.3, 0.07, and 1.3 cm rainfall on 1, 2, and 3 June, respectively.

The data from the 1995 field test (Table 4) show a phenomenon similar to the laboratory bioassays (Table 1), i.e., a high percentage of tarnished plant bugs treated with *B. bassiana* died from infections by 7 to 8 days after exposure to *B. bassiana*. Future research should focus on when infected tarnished plant bugs cease damaging plants.

These tests indicate that *B. bassiana*, either ARSEF 3769 or Mycotrol WP, could be an effective control agent for *L. lineolaris* in cotton or other crops if rapid kill (< 5 days) is not an important factor. There may be situations, such as in trap crops that are breeding/holding areas for *L. lineolaris*, or in wind strips, conservation areas, and weedy field borders, in which killing tarnished plant bugs in 5 to 8 days is satisfactory. If further research demonstrates that feeding is significantly reduced in infected *L. lineolaris*, this would add to the potential of *B. bassiana* for biological control of tarnished plant bugs. Snodgrass and Elzen (1994) applied another commercial product, Naturalis-L, which contains *B. bassiana* conidia, to cotton for *L. lineolaris* control and found that the product was moderately effective in controlling nymphs but was ineffective against adult tarnished plant bugs. However, the formulation and conidial concentrations of Naturalis-L are not directly comparable to the fungal formulations used in our tests.

The day 5 mortality (Table 4) shows that combining Provado with *B. bassiana* significantly increased mortality in exposed insects above that of either material alone. The reasons for this are unclear. Research on termites indicated that imidacloprid altered termite grooming behavior, increasing the susceptibility of the termites to fungal pathogens (Boucias et al. 1996). Such a behavior modifying mechanism could influence mobility or grooming of *L. lineolaris*. Recent research in Florida showed that mortality and mycoses in citrus root weevil were higher among larvae exposed to both fungi and imidacloprid than separate exposure. Imidacloprid stimulated an increase in the number of *Metarhizium anisopliae* Sorokin conidia germinating on the larvae. In addition, imidacloprid-treated larvae were less mobile in the soil thereby reducing their ability to remove attached conidia through normal activity in soil (E. D. Quintela and C. W. McCoy, University of FL, Lake Alfred, FL, unpublished data). If the economics are favorable, mixtures of Mycotrol and imidacloprid may provide better control of tarnished plant bugs and other insect pests than either material alone.

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