THE EFFECTS AND PERSISTENCE OF THE FUNGUS *BEAUVERIA BASSIANA* (MYCOTROL) AND IMIDACLOPRID (PROVADO) ON TARNISHED PLANT BUG MORTALITY AND FEEDING

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Abstract

The effectiveness of Mycotrol WP and Provado 1.6F were tested alone and in combination to control adult tarnished plant bug. Mycotrol WP contains the natural insect pathogen *Beauveria bassiana* which can infect a wide host range. Provado 1.6F contains imidacloprid which possesses activity against sucking insects. When Mycotrol and Provado were applied together at half of the maximum recommended rate for both insecticides, control was equal to that of Provado alone at maximum rate and greater than Mycotrol alone at maximum rate. Provado significantly increased tarnished plant bug mortality for at least 48 hours after spray. Mycotrol significantly increased mortality for 24 hours after spraying. Results from feeding trials suggested that plant bugs exposed to Provado showed an immediate decrease in feeding while those treated with Mycotrol only decreased feeding seven days after exposure.

Introduction

The tarnished plant bug (TPB), *Lygus lineolaris* (Palisot de Beauvois), is a serious pest of cotton and caused an estimated loss of 240,134 bales in the United States in 1995 (Williams 1996). TPB reduce yield by damaging bolls and feeding on small squares and terminals causing them to abort (Pack and Tugwell 1976, Tugwell et al. 1976, Hanny et al. 1977). Although effective control of TPB may be obtained through use of traditional pesticides containing carbamates, organochlorines, organophosphate, or pyrethroids (Pankey et al. 1996), resistant populations of TPB have been found in the Midsouth (Snodgrass and Scott 1988, Snodgrass 1994, Snodgrass and Elzen 1995) and early treatment with these insecticides can have season-long consequences on beneficial arthropods (Layton 1995).

Mycotrol WP (Mycotech, Butte, Montana) contains the fungus *Beauveria bassiana* (Strain GHA). This fungus is a naturally occurring pathogen of many different insects (Lipa 1963, Fargues and Remaudiere 1977). When Mycotrol is sprayed onto a field, billions of spores are distributed per acre. Spores infect insects by penetrating the exoskeleton and begin to multiply inside the insect causing death in 5 to 12 days (Fargues et al. 1994, Ingles et al. 1995). The fungus will emerge on the outside of the dead insect and disseminate new spores into the environment (Ingles et al. 1996). Since Mycotrol contains a living organism, it is possible that the fungus may recycle through the field if conditions were favorable.

Imidacloprid is the active ingredient in Provado 1.6F (Bayer Agricultural Division, Atlanta, Georgia). Imidacloprid is part of a new class of insecticides known as chlornicotinyls which possess activity against sucking insects such as aphids, whiteflies, and *Lygus* bugs (Elbert et al. 1990). Teague and Tugwell (1996) found that sub-lethal doses of Provado caused TPB to feed at a reduced rate. Some new research indicates that Provado enhances the pathogenicity of some fungal pathogens, including *Beauveria bassiana* (Quintela and McCoy 1997).

Materials and Methods

Sources of Materials

Provado 1.6F was obtained from Bayer Agricultural Division. Mycotrol WP(9504) was obtained from Mycotech. The number of spores per gram in Mycotrol WP was verified using a hemacytometer and phase microscope (Cantwell 1970). Spore viability was verified by plating 100 µl of the spore solution diluted to 1×10^7 spores per ml on each of three 9 cm Petri dishes containing SDAY media and held in the dark at 25^0C. After 24 hours, a drop of lactophenol-acid fuchsin stain and coverslip were placed on each dish. The number of germinated versus non-germinated spores were counted in five fields of view at 400X under a phase microscope for each plate and used to calculate mean germination rate. To determine how well Mycotrol survived over time, this procedure was also performed with a sample of Mycotrol WP which had been stored at 4^0C (40^0F) for one year.

Field trials

For all field experiments, adult mixed age TPB were collected from flowering mustard planted in cotton at the University of Arkansas Cotton Branch Experiment Station in Marianna, AR. Nylon tulle (1 mm^2 mesh) sleeve cages 3 ft long by 1 ft diameter placed over the first 6 terminal nodes of flowering cotton plants were used in all trials. Three days after treatment (DAT), TPB were collected from the cages. Live TPB were placed in individual 30 ml plastic cups with a 2.5 cm piece of fresh green bean and monitored for mortality 3, 5, and 7 DAT. TPB cadavers were placed in sporulation chambers (9 cm Petri dishes with moist filter paper) sealed with Parafilm and examined after 1 wk to assess mycoses. ANOVA and mean separation by LSD (α=0.05) were performed using the arcsine/square root of the proportion dead from each cage.

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Mortality test

The mortality test was performed on cotton, variety DPL 51, planted 17 May 1996 at the University of Arkansas Cotton Branch Experiment Station, Marianna, AR. A randomized complete block design was used with 4 blocks and 3 cages per treatment within a block with 15 TPB per cage. TPB were placed in cages immediately before spraying took place. Treatments were applied 9 July at 15 gpa with a 0.04% Kinetic solution as the carrier using a hand held CO_2 powered boom sprayer at 35 psi with Twin Jet 8002 VS nozzles. Treatments were as follows: Control (0.04% Kinetic solution), Mycotrol WP 1 lb/acre (M Full, highest recommended rate), Provado 0.047 lbs AI /acre (P Full, highest recommended rate), Mycotrol WP 1 lb/acre + Provado 0.047 lbs AI /acre (M+P Full), Mycotrol WP 0.5 lb/acre + Provado 0.024 lbs AI /acre (M+P Half), Mycotrol WP 0.25 lb/acre + Provado 0.012 lbs AI /acre (M+P Quarter).

Persistence test

The persistence test was performed on cotton, variety SureGrow 501, planted 20 May 1996 at the University of Arkansas Agricultural Research and Extension Center, Fayetteville, AR. Adult TPB were collected 16 July from flowering mustard planted in cotton at the University of Arkansas Cotton Branch Experiment Station, Marianna, AR and transported to Fayetteville. A randomized complete block design was used with 4 blocks and 3 cages per treatment within a block with 5 TPB per cage. Treatments were applied 17 July at 15 gpa with 0.04% Kinetic solution as carrier using a CO_2 powered backpack sprayer at 35 psi with Twin Jet 8002 VS nozzles. Treatments were as follows: Control (0.04% Kinetic solution), Mycotrol WP 1 lb/acre (M Full, highest recommended rate), Provado 0.047 lbs AI /acre (P Full, highest recommended rate), Mycotrol WP 0.5 lb/acre + Provado 0.024 lbs AI /acre (M+P Half), Mycotrol WP 0.25 lb/acre + Provado 0.012 lbs AI /acre (M+P Quarter). TPB were placed in cages immediately before spraying took place and additional replicates were placed in the field 12, 24, and 48 h after spray.

Feeding Study

Feeding studies were performed in conjunction with the persistence study. Additional cages of 5 TPB were placed in the field before spraying and removed after twelve hours of exposure. Feeding was monitored using the procedure outlined in Teague and Tugwell (1996). Twelve hours after treatment, live TPB were placed in individual petri dishes containing a 1 cm^3 of florist foam containing a 10% sucrose solution with green marker dye (McCormick's Green Food Coloring). Mortality and the number of fecal specks (frecks) were monitored 3, 5 and 7 days after treatment. The change in the mean number of frecks per day was compared using ANOVA and LSD (α=0.05). Dead TPB were placed in sporulation chambers and monitored for mycoses as above.

Results and Discussion

Mycotrol WP from 1996 contained 3.9 x 10^{10} spores/gram with 98.2% germination (Table 1). A chi squared test showed that there was no significant difference (χ^2 =13.4, df = 9) between the observed values for spores per gram and the expected value of 4.4 x 10^{10} spores/gram. The 1995 Mycotrol contained 3.3 x 10^{10} spores/gram with 99.1% germination. This shows that unused Mycotrol WP can be stored for at least one year and still retain excellent viability.

Mortality test

The results of the mortality test (Table 2) showed that mortality was greatest in the treatments which contained Provado. Using half of the maximum recommended rate of both materials induced the same initial mortality as the high rate of Provado alone and greater mortality than the high rate of Mycotrol alone. Low incidence of mycoses (Table 3) in treatments containing Mycotrol (only 52% in the full rate of Mycotrol) may have been the result of desiccation of the cadavers and subsequent death of the *B. bassiana* in the field. Steinkraus (1996) found that there was a strong interaction between imidacloprid and Mycotrol which increased TPB mortality. It is possible that the differences in the strength of the interaction in his study (Steinkarus 1996) and the present study arose predominately from differences in environmental conditions. If this is the case then it appears that this combination of pesticides would be best utilized early in the season when cooler temperatures and higher humidity were available.

Persistence tests

The results show that all treatments significantly increased TPB mortality for at least 24 hours after application, but only those treatments containing Provado lasted 48 hours (Table 4). Provado Full rate induced statistically greater mortality than the rest of the treatments until after the 12 hour sample. The mortality induced by (M+P) Half and (M+P) Quarter decreased in the 12 hour sample and rebounded by 24 hours suggesting that environmental variation may have a large influence the efficacy of these insecticides. During the hot, dry conditions prevalent from 17 to 19 July 1996, Mycotrol significantly increased TPB mortality for 24 hours but not 48 hours after initial application. The presence of cadavers which exhibited *B. bassiana* in the Mycotrol treatments 48 hours after treatment (Table 5) suggests that some infection of TPB still occurred after 24 hours but mortality was not elevated above background levels. Ingles et al. (1996) found that *B. bassiana* infected grasshoppers were able to reduce and sometimes cure infection by basking and increasing their temperature. It is possible that high temperatures during the mid and late summer would reduce the effectiveness of *B. bassiana* on tarnished plant bugs as well.

Feeding trials

The most severe and consistent initial reduction in feeding occurred in the treatments containing Provado (Table 6). TPB exposed to *B. bassiana* did not alter feeding rate early in the infection stage but did reduce feeding as the infection progressed. Fargues et al. (1994) found that Colorado potato beetles (*Leptinotarsa decemlineata* Say) increased feeding when first exposed to *B. bassiana* but then decreased feeding as the infection progressed. It is possible that this increased feeding also occurred in our study but was not statistically observable with our methods.

Conculsions

The results of this study suggest that the product Mycotrol WP can be stored from one season to the next at 4^0C with little or no loss of viability of spores. We also suggest that the data show that a combination of Mycotrol and Provado at half the recommended maximum rate per acre used early in the season may provide adequate control of tarnished plant bugs and have less impact on beneficial arthropods in the field. Mycotrol increased mortality for at least 24 hours after spray while Provado increased mortality for at least 48 hours, if not longer. Feeding studies indicated that an immediate reduction in feeding was caused by exposure to Provado and later reduction in feeding was caused by Mycotrol. This suggests that using the two chemicals in combination would reduce feeding and damage throughout the time necessary for infection to kill the insect.

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Table 1. Mean number and viability of *Beauveria bassiana* spores contained in Mycotrol WP from 1995 and 1996.

Year	Number of Spores (spores/gram)	Mean Percentage Spore Viability
1996[1]	3.9×10^{10}	98.2
1995[2]	3.1×10^{10}	99.1

[1]Number of spores per gram was not significantly different than the expected value of 4.4×10^{10} spores/gram (χ^2 analysis, P=0.05)
[2]Stored 1 year at 4^0C before viability test

Table 2. Mortality of tarnished plant bug adults in a 9 July 1996 field test after treatment with Mycotrol WP (*B. bassiana*) and Provado 1.6F (imidacloprid) in cotton at the Marianna Cotton Branch Field Station, Lee County, Arkansas.

Treatment	Rate of Mycotrol (lb/acre)	Rate of Provado (lbAI/acre)	Mean Percentage Mortality Days After Treatment 3	5	7
(M+P) Full	1	0.047	68.6a	73.5a	74.5a
Provado (P) Full	0	0.047	64.6a	68.7a	69.3a
(M+P) Half	0.5	0.024	65.7a	67.2a	68.7a
(M+P) Quarter	0.25	0.012	49.0b	51.8b	54.9b
Mycotrol (M) Full	1	0	19.3c	25.8c	43.8b
0.04% Kinetic	0	0	10.9d	12.8d	17.8d

Means with the same letter in a column are not significantly different. LSD, Alpha = 0.05

Table 3. Percentage of tarnished plant bug adults (TPB) cadavers in a 9 July 1996 field test which exhibited *B. bassiana* mycoses.

Treatment	Rate of Mycotrol (lb/acre)	Rate of Provado (lbAI/acre)	Percentage of Dead TPB Exhibiting *B. bassiana* Mycoses
(M+P) Full	1	0.047	0.0
Provado (P) Full	0	0.047	0.0
(M+P) Half	0.5	0.024	2.0
(M+P) Quarter	0.25	0.012	0.0
Mycotrol (M) Full	1	0	52.0
0.04% Kinetic	0	0	0.0

Table 4. Mortality of tarnished plant bug adults seven days after treatment in a 17 July field test in cotton at the Fayetteville Agricultural Research and Extension Center, Washington County, Arkansas.

Treatment*	Mean Percentage Mortality When TPB Placed in Field (hours after spray) 0	12	24	48
Provado (P) Full	93.3a	76.9a	70.0a	57.6a
(M+P) Half	80.6b	55.1b	68.9a	36.3ab
(M+P) Quarter	73.7b	33.3bc	63.1a	34.2ab
Mycotrol (M) Full	65.0b	39.9c	40.0b	20.1bc
Control	8.9c	16.7c	16.7c	16.1c

Means with the same letter in a column are not significantly different. LSD, Alpha = 0.05
*All treatments based on a Provado Full = 0.047 lbAI/acre and Mycotrol Full = 1 lb/acre

Table 5. Percentage of tarnished plant bug adults (TPB) cadavers in a 9 July 1996 field test which exhibited *B. bassiana* mycoses.

Treatment*	Percentage of Dead TPB Exhibiting *B. bassiana* Mycoses When TPB placed in field (hours after spray) 0	12	24	48
Provado (P) Full	0.0	0.0	4.1	0.0
(M+P) Half	9.6	8.3	9.0	8.3
(M+P) Quarter	29.2	25.0	4.2	0.0
Mycotrol (M) Full	86.1	66.7	4.7	12.5
Control	0.0	0.0	8.3	0.0

*All treatments based on a Provado Full = 0.047 lbAI/acre and Mycotrol Full = 1 lb/acre

Table 6. Feeding rate of tarnished plant bug adults as represented by the number of fecal specks (frecks) produced per day.

Treatment*	Mean Freck Production Rate (frecks/day) Between Sampling Periods Days After Treatment 3	5	7
Provado (P) Full	0.52a	0.57a	0.20ab
(M+P) Half	0.61a	0.59a	0.11a
(M+P) Quarter	0.67a	0.61a	0.30b
Mycotrol (M) Full	2.02b	0.96b	0.28ab
Control	1.97b	01.08b	0.75c

Means with the same letter in a column are not significantly different. LSD, Alpha = 0.05
*All treatments based on a Provado Full = 0.047 lbAI/acre and Mycotrol Full = 1 lb/acre