EFFECT OF *BEAUVERIA BASSIANA* FUNGUS ON THE BOXELDER AND RED SHOULDERED BUGS (HEMIPTERA: RHOPALIDAE)

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Abstract

Laboratory studies were initiated to assay the efficacy of BioCide-TRF™ (Mycotrol GH-ES) *Beauveria bassiana* Vuill. on two nuisance pests of the family Rhopalidae that frequently take winter protection in and around urban and suburban buildings. Micro-application treatments (2.5 μl droplet) of 6.5×10^5 conidia per insect, applied to the dorsal prothorax of the red shouldered bug, *Jadera haematoloma* (Herrich-Schaeffer), provided 100% control of adults in 8 d with 84% of the dead bugs expressing mycelia in that time period. Lower dosages did not provide significant mortality at 5 d, but by 8 d, 32-40% control was provided by the 4.0×10^4 and 1.6×10^5 conidia treatments. All adults of the boxelder bug, *Boisea trivittatus* (Say), exposed to treated leaves and seeds with the 1:100 dilution dose of *Beauveria* were killed within 7 d and 66.7% of the dead bugs expressed mycelia within 8 d. Significant mortality (83.3, 76.7 and 73.3%) was also provided by the 1:300, 1:500 and 1:1,000 dilution rates, respectively. This race of *B. bassiana* shows promise for control of both of these annual nuisance pests.

Key Words: *Boisea trivittatus, Leptocoris, Jadera haematoloma*, *Sapindus, Acer*

Resumen

Se efectuaron estudios de laboratorio para probar la eficacia de Biocide-TRF (Mycotrol GH-ES) *Beauveria bassiana* Vuill. en dos molestas plagas de la familia Rhopalidae que toman protección en el interior y exterior de edificios urbanos y suburbanos. La microaplicacion de una gota de 2.5 μl conteniendo 6.5×10^5 conidios/insecto colocada en el protorax dorsal del insecto de lomo rojo, *Jadera haematoloma* (Herrich-Schaeffer) controló 100% de los adultos en 8 días. El 84% de los insectos muertos mostraron desarrollo de micelios. Las dosis de 4.0 y 1.6×10^5 conidios/insecto lograron un control de 32 a 40% a los 8 días. Todos los adultos del insecto *Boisea trivittatus* (Say) expuestos a hojas y semillas tratadas con una dilución de 1:100 de *Beauveria* murieron en 7 días y 66.7% de los insectos muertos mostraron desarrollo de micelios en 8 días. Las diluciones de 1:300, 1:500, y 1:1000 también causaron mortalidad significativa (83.3, 76.7, y 73.3%, respectivamente). Esta raza de *B. bassiana* muestra potencial para el control de las dos plagas estudiadas.

The family Rhopalidae, or scentless plant bugs, is small but contains several species that quite often become serious nuisance pests in the fall as they take winter protection in and around urban and suburban buildings. The boxelder bug (BEB) (formerly in the genus *Leptocoris* Hahn), and the red shouldered bug (RSB), each feed on the fruits of their respective hosts throughout their nymphal and adult development. Damage by both species usually occurs early in the season as the overwintering adults feed on the developing buds. This feeding may distort the foliage and cause the plants to be bushy.

RSB is a household nuisance pest across the range of its primary breeding host, Chinaberry, *Sapindus drummondii* Hook & Arn. When Chinaberry trees are present in the landscape, this pest often reaches high populations on the developing yellow berries. As the nymphs and adults mature, large numbers are often seen migrating up and down the tree trunks, across the lawn and landscape, and into houses and other nearby buildings throughout the summer and early fall. This migration into buildings takes place both during the breeding period and later in search of overwintering sites. RSB has a known distribution including AL, AZ, CA, CO, FL, IL, KS, MO, OK, the West Indies and Mexico to Columbia and Venezuela, but its biology is poorly understood (Henry 1988, Arnold et al. 1992).

BEB is a common household nuisance pest which also leaves its host plant in late fall to seek overwintering sites in and around buildings. Their annual invasion of houses on warm days in late fall, winter and spring in search of overwintering sites is well documented (Arnold et al. 1992, Ebeling 1978, Johnson & Lyon 1992). This insect breeds on the boxelder tree, *Acer negundo* L., and it also feeds and develops large populations on other *Acer* spp. BEB is known from central and eastern Canada throughout the eastern United States and west to MT, NV and CA (Dreistadt et al. 1994, Henry 1988). Its life history and behavior has been studied by Smith and Shepherd (1937) and Tinker (1952). Wheeler (1982) provides an extensive review of the literature on its economic importance and biology.

Several biocontrol products, containing formulations of *Beauveria,* have been used successfully against other families of Hemiptera in other countries around the world and a review is presented by McCoy et al. (1985). In the Peoples Republic of China, certain strains of *B. bassiana* are produced and used for control of the European corn borer, *Ostrinia nubilalis* (Hubner). In several eastern European countries, *Beauveria* is mass produced for use against the Colorado potato beetle, *Leptinotarsa decemlineate* (Say).

In the U.S., various strains of *B. bassiana* have been widely tested against several primary pests including the Colorado potato beetle (Watt & Lebrun 1984); lygus bugs, *Lygus* spp., and several stored products pests (Dunn & Mechalas 1963); chinch bugs, *Blissus leucopterus leucopterus* (Say) (Ramoska & Todd 1985); green peach aphid, *Myzus persicae* (Sulzer) (Kish et al. 1994); Russian wheat aphid, *Diuraphis noxia* Kurdyumov, (Wang & Knudsen 1993); pear psylla, *Cacopsylla pyricola* (Foerster) (Puterka et al. 1994) and the red imported fire ant, *Solenopsis invicta* Buren (Oi et al. 1994). Naturalis® L, a strain of *B. bassiana,* has been evaluated in cotton and exhibited activity in tests against boll weevil, *Anthonomous grandis grandis* Boheman; tarnished plant bug, *Lygus lineolaris* (Palisot de Beauvois); cotton fleahopper *Pseudatomoscelis seriatus* (Reuter) and silverleaf whitefly, *Bemisia argentifolii* Bellows & Perring (Knauf 1995).

The purpose of this study was to evaluate the BioCide-TRF™ (Mycotrol GH-ES) strain of *B. bassiana* as a low-impact environmentally-sound control measure for RSB and BEB for use in urban landscapes.

Materials and Methods

RSB Experiment:

For this study, RSB adults were collected as they migrated up and down the trunk of several Chinaberry trees on the campus of the Texas A&M University Research and

Extension Center at Dallas, TX. Adults were brushed into 3.79 liter (1 gal) ice cream containers fitted with 15 cm diam. funnel modified lids to prevent escapes. They were held for 24 h; only adults that appeared healthy after 1d in captivity were used for the study.

The RSB Experiment was set up on 28 June, 1996. Captive adults were divided into units of 5 randomly-selected adults in 4-dram shell vials with stoppers. Vials were randomly assigned to treatments and emptied into 9 cm diam × 15 mm deep plastic petri dish feeding chambers that were labeled for the respective treatments in a randomized complete block design with 5 reps. Each chamber was provided with two 7.5 cm diam filter papers moistened with water to provide constant, high humidity during the study. Additionally, 2 chinaberry fruits were added to each chamber as a food source during the study.

Four dilutions of BioCide-TRF™ (Mycotrol GH-ES) *B. bassiana* (2.11×10^{13} conidia/liter) (2.0×10^{13} conidia/qt) were compared with chlorpyrifos (as a commercial standard) and an untreated check (rates listed in Table 1). The adults confined in each chamber were then anesthetized with CO^2 gas and a 2.5 µl droplet of the respective conidia dilution or chlorpyrifos solution was applied with a syringe to the dorsal prothorax of each of the five individuals. Silwet™ (silicon + polyether copolymer) (0.04%), a wetting agent, was added to each dilution to insure adherence to the normally hydrophobic cuticles of the insects. If a treated bug did not show complete recovery within 10 min, another adult was treated and replaced it. Treatments were observed daily for mortality; all dead or moribund insects were held for several days to confirm the fungus activity. The percent mortality was recorded at 5 and 8 d after treatment and confirmed as an expression of *Beauveria* mycelia from the joints of the dead insects. Fungal infection was confirmed from representative cadavers by culturing on Saubaroud maltose agar (SMA) (Difco, Detroit, MI) petri plates in the lab.

TABLE 1. MORTALITY OF ADULT RED SHOULDER BUGS TREATED WITH DILUTIONS OF BIOCIDE (MYCOTROL GH-ES 32% STRAIN OF *BEAUVERIA BASSIANA*) CONTAINING 2.11×10^{13} CONIDIA/LITER, DALLAS, TX, JULY 1996 (5 REPS).

		5 days[b]		8 days[b]	
Treatment	Conidia[a]	Mortality (%)	Mycelia (%)	Mortality (%)	Mycelia (%)
BioCide 32%	6.5×10^5	56 a[c,d]	12 a[c,d]	100 a[c,d]	84 a[c,d]
BioCide 32%	1.6×10^5	4 b	0 b	32 cd	8 b
BioCide 32%	4.0×10^4	16 b	0 b	40 c	4 b
BioCide 32%	1.0×10^4	0 b	0 b	12 d	0 b
Chlorpyrifos 6.6% EC	1.2 g[e]	64 a	0 b	76 b	0 b
Untreated Check	—	10 b	0 b	10 d	0 b

[a]2.5 µl of each concentration was applied to the dorsal prothorax of each adult bug.
[b]Adult mortality and % expression of fungal mycelial growth at days post inoculation.
[c]Analysis was made on arcsine transformation of the data: untransformed data is presented.
[d]Means in a column not followed by the same letter are significantly different by Waller-Duncan k-ratio t-test (k = 100) (P = 0.05).
[e]Rate as 1.2 g ai/liter (1 lb ai/100 gal).

BEB Experiment:

Adults for this study were collected from a developing population (ca. 50% adults) of boxelder bugs on a bigtooth maple, *Acer grandidentatum* Nutt., that had developed a heavy seed set, also in the landscape at the TAMU-REC, Dallas, TX. Leaves and seed were also collected from this host tree to be used as a food source during the study. A second method of exposure to the *Beauveria* was also investigated in this study in the lab. One leaf and 3 seeds were dipped in the appropriate dilutions (Table 2) of BioCide™ (Mycotrol GH-ES) *B. bassiana* (2.11×10^{13} conidia/liter), allowed to air dry and placed on two water-saturated filter papers (for constant humidity) in each feeding chamber for exposure to the *Beauveria*. Silwet was added to the dilutions as above. On 4 November 1996, 5 randomly-selected adults, that had been held for 1d as above, were introduced into each 9 cm diam × 15 mm deep plastic feeding chambers labeled for the respective treatment in a randomized complete block design with 6 reps. Both a water + Silwet dipped and an untreated check were included. Treatments were observed daily for mortality; all dead insects were held for several days to confirm the fungal infection and cultured the same as with the RSB.

Data were subjected to analysis by the General Linear Model procedure and treatments were separated by Waller-Duncan k-ratio t-test (k = 100) (P = 0.05) (SAS Institute 1985). Mortality data were transformed by arcsine before analysis, but untransformed means are presented here.

RESULTS AND DISCUSSION

RSB Experiment:

Results of the study are summarized in Table 1. All of the adults treated with the 6.5×10^5 conidia per insect dose of *Beauveria* were killed and 84% of the dead bugs expressed mycelia within 8 d, and this treatment provided significantly greater mortality ($P < 0.05$) than the other treatments, including chlorpyrifos (76%). The lower dosages did not provide significant mortality at 5 d, but by 8 d, 32-40% control was provided by the 4.0×10^4 and 1.6×10^5 conidia doses, respectively. The chlorpyrifos standard, however, provided significantly higher mortality than either of these lower rates.

BEB Experiment:

Results of this study are summarized in Table 2. The 1:100 dilution of *Beauveria* applied to leaves and seeds of *Acer grandidentatum* killed 100% of the BEB within 7 d and 66.7% of the dead bugs expressed mycelia within 8 d. Significant mortality at 8 d (83.3, 76.7 & 73.3%) was also provided by the 1:300, 1:500 and 1:1,000 dilution rates, respectively. The 2 lowest dilution rates tested were not significantly better than the untreated checks. Also, no difference was recorded between the water dipped + Silwet and the other untreated check. As a general trend, it took longer to kill the BEB, the more dilute the *Beauveria* treatment.

The BioCide-TRF™ (Mycotrol GH-ES) strain of *B. bassiana* shows promise for control of each of these annual nuisance pests. Both methods of application yielded a good separation among the rates tested. For the micro-application directly to the insects, the separation was between 6.5×10^5 and 1.6×10^5. With treatments applied to the substrate (air dried), a significant separation occurred between dilutions of 1:100 and 1:300. The primary separation in efficacy occurred between dilutions of 1:1,000 and

TABLE 2. MORTALITY OF ADULT BOXELDER BUGS CONFINED ON BIGTOOTH MAPLE LEAF AND SEEDS THAT WERE TREATED WITH DILUTIONS OF BIOCIDE (MYCOTROL GH-ES 32% STRAIN OF *BEAUVERIA BASSIANA*) (CONTAINED 2.11 × 10^{13} CONIDIA / LITER), DALLAS, TX, JULY 1996 (6 REPS).

		5 day[a]		7 day[a]	8 days[a]		9 day[a]
Treatment	Dilution	Mort (%)	Mycelia (%)	Mort (%)	Mort (%)	Mycelia (%)	Mort (%)
BioCide 32%	1:100	73.3 a[b,c]	60.7 a[b,c]	100 a[b,c]	100 a[b,c]	66.7 a[b,c]	100 a[b,c]
BioCide 32%	1:300	6.7 b	3.3 b	63.3 b	76.7 b	16.7 b	83.3 b
BioCide 32%	1:500	23.3 b	13.3 b	53.3 bc	56.7 b	33.3 ab	76.7 b
BioCide 32%	1:1,000	3.3 b	0 b	33.3 cd	56.7 b	20.0 b	73.3 b
BioCide 32%	1:10,000	0.0 b	0 b	10.0 d	26.7 c	0 b	26.7 c
BioCide 32%	1:100,000	3.3 b	0 b	6.7 d	6.7 c	0 b	6.7 c
Untreated dipped	—	13.3 b	0 b	16.7 d	23.3 c	0 b	26.7 c
Untreated	—	13.3 b	0 b	13.3 d	16.7 c	0 b	23.3 c

[a]Adult mortality and % expression of fungal mycelial growth at days post inoculation.
[b]Analysis was made on arcsine transformation of the data: untransformed data is presented.
[c]Means in a column not followed by the same letter are significantly different by Waller-Duncan k-ratio t-test (k = 100) (P = 0.05).

1:10,000. These results should serve as a guide in determining field treatment rates. Field trials in the landscape are planned to confirm these results and determine the dilution rates required for control under field conditions.

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