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Laboratory Assessment of *Beauveria bassiana* (Hypocreales: Clavicipitaceae) strain GHA for Control of *Listronotus maculicollis* (Coleoptera: Curculionidae) Adults

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ABSTRACT Bioassays were designed to evaluate *Beauveria bassiana* (Balsamo) Vuillemin strain GHA against *Listronotus maculicollis* (Kirby) adults. *B. bassiana* and its "inert" carrier oil in the product BotaniGard and the inert carrier oil alone provided 99 and 96% mortality, respectively, in petri dish assays 1 d after treatment when applied in 1 ml water. When the same treatments were applied in 0.5 ml of carrier water, mortality was only 1.4 and 0.7%, respectively, 1 d after treatment. After 10 d in petri dishes, *B. bassiana* and its inert carrier oil and the inert carrier oil alone applied in 0.5 ml water showed 77 and 9% mortality, respectively. When one-tenth the label dosage of *B. bassiana* and inert carrier oil was combined with neonicotinoids applied in 1 ml water, there were significant increases in weevil mortality over the neonicotinoids alone 1 d after treatment. When 88.7% of one-tenth the label dosage of inert carrier oil alone was combined with neonicotinoids clothianidin, imidacloprid, and dinotefuran applied in 1 ml water, there was also a significant increases (38%) with clothianidin in weevil mortality over clothianidin alone 1 d after treatment. *B. bassiana* and its inert carrier oil provided 28, 50, and 78% mortality at the highest label dosage and 47, 76, and 89% mortality at 4× the highest label dosage in turf plug assays at 7, 10, and 14 d after treatment. Addition of 5 or 20% MycoMax (a nutrient source for *B. bassiana*) did not significantly increase mortality.

KEY WORDS *Beauveria bassiana*, *Listronotus maculicollis*, Curculionidae, neonicotinoid

Larvae of *Listronotus maculicollis* (Kirby) (Coleoptera: Curculionidae) are the most destructive insect pests of *Poa annua* L. (Poales: Poaceae) on golf courses in the northeastern United States (Vittum et al. 1999). This insect species was first seen damaging turfgrass in Connecticut in 1931 and by the late 1950s and early 1960s was responsible for severe damage on golf courses in the state (Britton 1932, Tashiro 1976). Adult *L. maculicollis* chew notches on grass blades at the juncture of leaves and stems. Adult damage is not as severe as larval feeding. Larval feeding can result in extensive turf damage and death, as they feed at the plant crown. Where larval densities exceed 450 per 929 cm^2 (1 $foot^2$), injury to golf course greens, collars, and fairways is common (Watschke et al. 1994). Instars 1–3 feed inside plant stems while 4th and 5th instars feed on plant crowns. There are normally two to three generations of *L. maculicollis* per year in the northeastern United States.

Fourth-generation pyrethroids provided excellent control of weevils in the 1990s and early 2000s. These products were principally used to target adult weevils, as they colonized turf after overwintering in areas surrounding tees, greens, and fairways. In 2005, the first indications of diminished pyrethroid effectiveness were reported (Vittum 2005). In 2009, the first study to confirm pyrethroid resistance was published (Ramoutar 2009a), and two subsequent studies (Ramoutar et al. 2009b, 2010) further confirmed pyrethroid resistance.

Alternative controls are needed to manage this serious pest. *Beauveria bassiana* (Balsamo) Vuillemin is an important biological control agent for many insect pests (Quintela and McCoy 1998, Fargues and Luz 2000, Furlong and Groden 2001). In this study, we evaluated a commercially available formulation of *B. bassiana* against *L. maculicollis* adults in petri dish assays and simulated field studies with turfgrass plugs both with and without neonicotinoid insecticides and a nutrient source for *B. bassiana* called MycoMax. We concentrated on adult control because instars 1–3 are protected inside plant stems and can only be controlled with systemic insecticides and by the time 4th and 5th instars emerge, the majority of damage has occurred. Furthermore, adult population densities are more readily monitored than larval stages.

Materials and Methods

Petri Dish Assays. Adult weevils were collected from several golf courses and placed on 9-cm-diameter filter paper discs in 100- by 15-mm petri dishes treated with various dosages of *B. bassiana* strain GHA and its "inert" carrier oil (BotaniGard, Laverlam Interna-

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tional, Butte, MT). Other treatments included the inert carrier oil alone, and neonicotinoid insecticides and combinations with BotaniGard and its inert carrier oil applied in 0.5 or 1 ml water. Controls consisted of treating filter paper discs with 1 ml of water and adding adult weevils. Petri dishes were wrapped in parafilm to maintain humidity and prevent weevils from escaping. Assays were rated for adult mortality for up to 10 d. In the first set of assays, we evaluated the highest label dosage of *B. bassiana* strain GHA (25.46 liters/ha) and the inert carrier oil at 25.46 liters/ha 1, 3, 5, 7, and 10 d after treatment. Inert carrier oil was obtained by first centrifuging BotaniGard at 8,820 rpm for 20 min. The supernatant carrier oil was then filtered 2× (Millex HV 0.45 μm then MillexVV 0.1 μm, EMD Millipore Corp., Billerica, MA) and used in experiments. Filtered inert carrier oil and BotaniGard were also plated on Sabouraud Dextrose Agar (SDA) plates incubated at 25°C and checked for fungal growth up to 10 d. Six separate experiments with weevils from three locations (Westerly, RI; Baltic and Norwich, CT) were combined for analysis (10 weevils per replicate, 14 replicates, 140 weevils for each treatment).

In a second assay, we evaluated three neonicotinoid insecticides—imidacloprid (Bayer Environmental Science, Research Triangle Park, NC), clothianidin (Valent U. S. A. Corp., Walnut Creek, CA), and dinotefuran (PBI–Gordon, Kansas City, MO)—at label dosages with and without one-tenth the label dosage of *B. bassiana* strain GHA and its inert carrier oil and 88.7% of one-tenth the label dosage of the inert carrier oil alone for adult mortality 1, 3, 5, and 7 d after treatment. One experiment was conducted with five replicates with weevils from Westerly, RI (10 weevils per replicate, 5 replicates, 50 weevils per treatment).

Turf Plug Assays. In another series of experiments, we evaluated the highest label dosage of BotaniGard (25.46 liters/ha); however, mortality was not high enough to be able to recommend it as a control option. Therefore, we compared the highest label dosage and 4× the highest label dosage (101.84 liters/ha) to explore a dosage response, and to determine whether mortality would be comparable with standard synthetic insecticides. Controls consisted of treating 5.72-cm-diameter turfgrass plugs with water only and adding 10 weevils. Turfgrass plugs were treated using a CO_2-powered sprayer equipped with an 8002EVS TeeJet nozzle (Spraying Systems Co., Wheaton, IL). After treatment, turfgrass plugs were placed in 147-ml plastic cups where the weevils were added and then contained with a 1-mm mesh screen. Three assays using weevils from two locations (Westerly, RI, and Norwich, CT; 10 weevils per plug, 4 replicates, 120 weevils per treatment) were rated for adult mortality 7, 10, and 14 d after treatment and combined for analysis.

In another set of turf plug assays, adult weevils (5 per plug, 4 replicates, 20 weevils per treatment) were collected from Westerly and Pawtucket, RI, and placed on turfgrass plugs treated with the highest label dosage (25.46 liters/ha) of *B. bassiana* strain GHA in its inert carrier oil both with and without 5 and 20% MycoMax (a sweet whey designed to be a nutrient source for *B. bassiana* obtained from Scott Costa, University of Vermont) in volumes of water equal to 815 liters/ha (2 gal/1,000 feet^2). Controls consisted of treating turfgrass plugs with water only and adding five weevils.

Statistical Analysis. Percent mortalities were analyzed by analysis of variance (ANOVA) followed by mean separation by Tukey's honestly significant difference (HSD) test (SAS Institute 2002–08).

Results

Petri Dish Assays. There was significant mortality of adults in petri dish assays at the highest BotaniGard label dosage and the inert carrier oil filtered 2× at the same dosage at 1 d ($F = 1701.20$; df = 4,52; $P < 0.01$), 3 d ($F = 1751.81$; df = 4,52; $P < 0.01$), 5 d ($F = 1170.16$; df = 4,52; $P < 0.01$), 7 d ($F = 397.87$; df = 4,52; $P < 0.01$), and 10 d ($F = 442.70$; df = 4,52; $P < 0.01$) after treatment when applied in 1 ml of water (Fig. 1). We

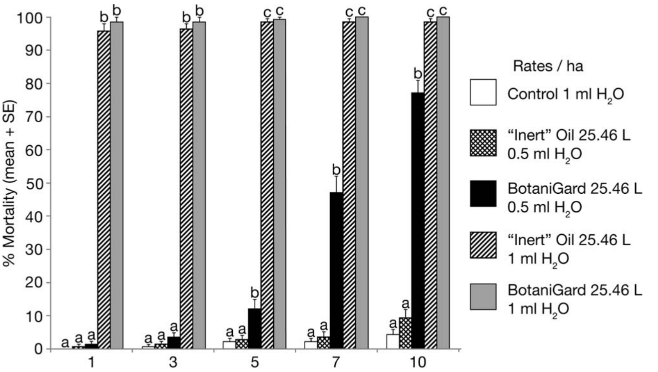

Fig. 1. Percent mortality (mean + SE) of adults at the highest BotaniGard label dosage (25.46 liters/ha) and its carrier oil applied in 0.5 and 1.0 ml of water vs. a water control in petri dish assays, 1, 3, 5, 7, and 10 d after treatment. Means followed by the same letter are not significantly different ($P = 0.05$, Tukey's HSD test).

Table 1. Percent mortality (±SE) of BotaniGard, "inert" oil, and clothianidin, imidacloprid, and dinotefuran alone and with BotaniGard and inert oil against *L. maculicollis* adults 1, 3, 5, and 7 d after treatment (DAT) in a petri dish assay

Treatment (rates a.i./ha)	Percent mortality (mean ± SE)			
	1 DAT	3 DAT	5 DAT	7 DAT
Control	2 ± 2a	2 ± 2a	6 ± 4a	24 ± 4a
BotaniGard (0.28 liters)	4 ± 2ab	8 ± 2ab	64 ± 7c	100 ± 0c
BotaniGard (2.87 liters)	100 ± 0e	100 ± 0d	100 ± 0d	100 ± 0c
Inert Oil (2.25 liters)	0 ± 0a	2 ± 2a	4 ± 2a	22 ± 5a
Clothianidin (448 g)	14 ± 4ab	84 ± 4d	100 ± 0d	100 ± 0c
Clothianidin (448 g) + BotaniGard (0.28 liters)	48 ± 14cd	100 ± 0d	100 ± 0d	100 ± 0c
Clothianidin (448 g) + Inert Oil (2.25 liters)	52 ± 10cd	100 ± 0d	100 ± 0d	100 ± 0c
Imidacloprid (448 g)	6 ± 4ab	30 ± 6bc	36 ± 5b	56 ± 13b
Imidacloprid (448 g) + BotaniGard (0.28 liters)	36 ± 8bc	56 ± 9c	90 ± 3d	100 ± 0c
Imidacloprid (448 g) + Inert Oil (2.25 liters)	28 ± 12abc	54 ± 13c	58 ± 11cb	80 ± 7bc
Dinotefuran (605 g)	6 ± 2ab	30 ± 7bc	66 ± 7c	86 ± 6c
Dinotefuran (605 g) + BotaniGard (0.28 liters)	74 ± 7de	90 ± 3d	100 ± 0d	100 ± 0c
Dinotefuran (605 g) + Inert Oil (2.25 liters)	30 ± 6abc	42 ± 8c	62 ± 6c	92 ± 4c

Means followed by the same letter are not significantly different ($P = 0.05$, Tukey's HSD test) 1 DAT: $F = 21.48$; df = 12,48; $P < 0.01$; 3 DAT: $F = 45.91$; df = 12,48; $P < 0.01$; 5 DAT: $F = 52.76$; df = 12,48; $P < 0.01$; 7 DAT: $F = 33.09$; df = 12,48; $P < 0.01$.

also demonstrated with petri dish assays that using 0.5 vs. 1.0 ml of application water with 16.19 μl BotaniGard or 16.19 μl inert carrier oil alone per 9-cm-diameter filter paper disc (=25.46 liters/ha) was the difference between low and high mortality, respectively (Fig. 1). A significant increase in mortality of weevils because of *B. bassiana* over the inert carrier oil alone began 5 d after treatment (12 vs. 3%, respectively) and differences between the two treatments continued to increase on days 7 (47 vs. 4%) and 10 (77 vs. 9%) after treatment (Fig. 1). In another assay, one-tenth the label dosage of *B. bassiana* began to show significant mortality starting on day 5 and was not statistically different from the full label rate by 7 d after treatment (Table 1). No fungal growth was observed on SDA plates streaked with inert carrier oil, whereas plates streaked with BotaniGard did show fungal growth during the 10-d examination period.

There was significant mortality of adults in petri dish assays with BotaniGard at the label dosage (25.46 liters/ha) and dinotefuran with one-tenth the label dosage of BotaniGard (2.54 liters/ha; $F = 21.48$; df = 12,48; $P < 0.01$) 1 d after treatment (Table 1). There was no significant difference in mortality of adults in petri dish assays with label dosages of clothinidin and imidacloprid with one-tenth the highest label dosage of BotaniGard (2.54 liters/ha) or 88.7% of the inert oil (2.25 liters/ha) contained in one-tenth the highest labeled dosage of BotaniGard 1 d after treatment in petri dish assays (Table 1). Again, the effect of *B. bassiana*, at one-tenth the label dosage began to show significant mortality on day 5 after treatment and increased to 100% mortality on day 7. When comparing clothianidin, imidacloprid, and dinotefuran alone, clothianidin caused significantly greater mortality than the other two neonicotinoids beginning on day 3 after treatment (Table 1).

Turf Plug Assays. When adults were placed on treated turfgrass plugs, there was significant mortality at the highest and 4× the highest label dosage at 7 d ($F = 25.19$; df = 2,22; $P < 0.01$), 10 d ($F = 52.93$; df = 2,22; $P < 0.01$), and 14 d ($F = 22.89$; df = 2,22; $P < 0.01$) after treatment (Fig. 2). Four times the highest label dosage caused faster and greater mortality (Fig. 2).

Addition of 5 or 20% MycoMax (an adjuvant providing sweet whey as a nutrient source for *B. bassiana*) did not significantly increase mortality at 7 d ($F = 1.12$; df = 2,14; $P = 0.35$), 10 d ($F = 2.96$; df = 2,14; $P = 0.08$), or 14 d ($F = 2.03$; df = 2,14; $P = 0.17$) after treatment.

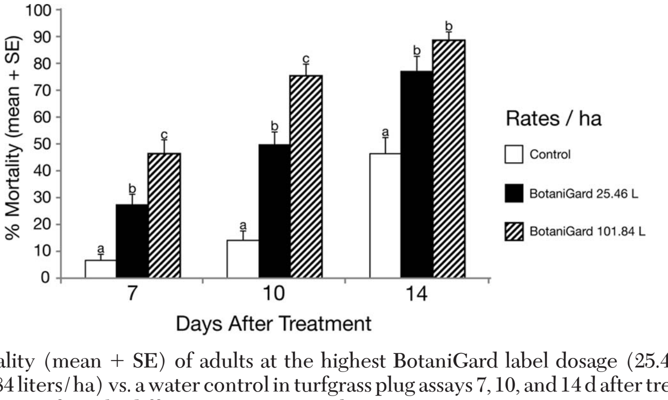

Fig. 2. Percent mortality (mean + SE) of adults at the highest BotaniGard label dosage (25.46 liters/ha) and 4× the highest label dosage (101.84 liters/ha) vs. a water control in turfgrass plug assays 7, 10, and 14 d after treatment. Means followed by the same letter are not significantly different ($P = 0.05$, Tukey's HSD test).

Discussion

Petri dish assays at the highest label dosage (25.46 liters/ha) and the inert carrier oil filtered 2× at that dosage were effective in causing mortality of adult weevils at 24 h when applied in 1 ml water. This indicates that mortality within 24 h was the result of the oil and not infection from *B. bassiana* strain GHA. When using oil formulations of entomopathogenic fungi, there needs to be assurance that insecticidal activity is not the result of the oil carrier (Goettel and Inglis 1997). Typical infection with entomopathogenic fungi such as *B. bassiana* will normally take several days to exert lethal effects, which we did notice beginning on day 5 or 7 in all three assays.

Cowles et al. (2000) used leaf dip bioassays with twospotted spider mites, *Tetranychus urticae* Koch, and demonstrated the toxicity of trisiloxane surfactants, also considered inert ingredients. Toxicity was influenced by the leaf dip method, which exaggerated the degree of wetting and indicated that high toxicity from surfactants was likely only in extremely wetted applications with high humidity. Although we were using inert carrier oil as a treatment and not a surfactant, there was high mortality associated with a higher degree of wetness. This suggests that the petri dish assay treatments of either BotaniGard and its carrier oil or the carrier oil alone using 1 ml of water may be effectively drowning the weevils similar to the activity of surfactants against *T. urticae* reported by Cowles et al. (2000).

The influence of moisture and humidity was also evident in the difference between treatments applied in volumes of 0.5 vs. 1.0 ml of water. The inert carrier oil was ineffective when applied with 0.5 ml of water where the petri dish was not as wet. However, when BotaniGard was applied in petri dishes in 0.5 ml of water, significant mortality was evident starting at 5 d and increased at 7 and 10 d after treatment. Therefore, humidity and moisture was still adequate when treatments were applied in 0.5 ml of water for *B. bassiana* to begin to infect and kill adult *L. maculicollis* 5 d after treatment. Other experimental work has shown that moisture was critical in effectiveness of *B. bassiana* strain GHA. Fargues and Luz (2000) found that the pathogenic activity of *B. bassiana* to *Rhodnius prolixus* Ståhl was highly dependent on the moisture conditions and to a lesser extent on the temperature conditions. Their results showed a critical threshold of relative humidity between 95.5 and 97%.

One of the most encouraging results was the effect of one-tenth the label dosage of BotaniGard or the inert carrier oil with neonicotinoids (imidacloprid, clothianidin, and dinotefuran). We noticed in other assays that neonicotinoids have a quick knockdown effect on adults; however, adults normally recover within 24 h. Clothianidin did cause significantly greater mortality than either imidacloprid or dinotefuran 3 d after treatment. Immobilizing adults for 24 h may allow *B. bassiana* to overcome the insects' defense mechanisms or the inert carrier oil is drowning adults. The highest label dosage of BotaniGard is the equivalent of 53,819 conidia/mm^2. This is a high rate when compared with other studies that demonstrated the effectiveness of 100 conidia/mm^2 against *Helicoverpa zea* (Boddie) and *Spodoptera exigua* (Hübner) larvae (Wraight et al. 2010) or 1,271 conidia/mm^2 vs. third-instar whitefly nymphs, *Trialeurodes vaporariorum* (Westwood) (Poprawski et al. 2000).

There was a significant difference in mortality between one-tenth the label dosage of BotaniGard and its inert carrier oil on day 5 after treatment with imidacloprid and dinotefuran. Furlong and Groden (2001) found that significant synergism occurred in all instances where Colorado potato beetle, *Leptinotarsa decemlineata* (Say), larvae were exposed to imidacloprid before or simultaneously with *B. bassiana* treatment. They suggested that the synergism probably involves changes in the insect's physiology that affects successful cuticular penetration or the initial proliferation of *B. bassiana* hyphal bodies within the host hemocoel. Quintela and McCoy (1998) found that the addition of imidacloprid to soil significantly impaired movement of larval *Diaprepes abbreviates* (L.). When either *B. bassiana* or *Metarhizium anisopliae* (Metschnikoff) Sorokin were applied with imidacloprid, mortality and mycosis increased significantly. Surfactants in formulations of synthetic insecticides may also increase infection by *B. bassiana.*

Data on how quickly mortality of adults is achieved are important in management decisions, as, if immediate control is needed to prevent damage, the highest label dosage of BotaniGard may not act quickly enough to prevent damage. When adults were placed on treated turfgrass plugs, there was significant mortality at the highest and 4× the highest label dosage of BotaniGard 7, 10, and 14 d after treatment. Four times the highest label dosage did cause significantly faster and greater mortality at 7 and 10 d after treatment. Eventually at 14 d after treatment, the highest label dosage did cause significant mortality that was not significantly different from the 4× label dosage. The addition of MycoMax, a sweet whey adjuvant designed as a nutrient source for insect pathogenic fungi, did not significantly increase adult mortality. These data demonstrate that *B. bassiana* either alone or combined with a neonicotinoid, a surfactant, or both, may have potential for control of *L. maculicollis* in the field.

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