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S.P. Wraight, M.E. Ramos

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Characterization of the synergistic interaction between *Beauveria bassiana strain* GHA and *Bacillus thuringiensis morrisoni* strain tenebrionis applied against Colorado potato beetle larvae

S. P. Wraight[1] and M. E. Ramos[2]

[1] USDA-ARS, Robert W. Holley Center for Agriculture and Health, Ithaca, NY 14853

[2] Formerly USDA-ARS, R.W. Holley Center, Ithaca, NY 14853

[*] Corresponding author: Stephen P. Wraight

USDA-ARS Robert W. Holley Center for Agriculture and Health, Ithaca, NY 14853

Phone: 607-255-2458, Email: steve.wraight@ars.usda.gov

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Highlights

- *B. bassiana* and *B. thuringiensis* were applied jointly vs. potato beetle larvae.
- Results indicated synergism of *B. bassiana* activity by *B. thuringiensis* toxinosis.
- Speed of kill by the combined pathogens was no greater than that of Bt alone.
- Consistent levels of synergism were observed under variable environmental conditions.
- Results underscored the strong complementary action of the two biocontrol agents.

Abstract

Studies were undertaken to further characterize the previously identified synergistic activity of *Bacillus thuringiensis*- and *Beauveria bassiana*-based biopesticides against Colorado potato beetle (CPB). A flowable concentrate of *B. thuringiensis morrisoni* strain tenebrionis (Bt) (Novodor® FC) and a wettable powder of *B. bassiana* strain GHA (Bb) (Mycotrol® 22WP) were applied against CPB larval populations infesting potato in field plots. Novodor FC and an oil-dispersion formulation of Bb (Mycotrol ES) were applied against second-instar CPB larvae on potted potato plants in greenhouse tests under low relative humidity (RH), variable-temperature conditions. Each pathogen was applied alone and in combination (tank-mixed) with the other pathogen. In the field tests, each biopesticide was also combined with the spray-carrier (formulation without active ingredient) of the other pathogen. Results from the greenhouse tests showed that under warm, dry conditions, low activity of Mycotrol was counterbalanced by high activity of the Novodor, and under cool, somewhat more humid conditions, low Novodor activity was balanced by high activity of Mycotrol, with the result being a constant level of synergism

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(CPB mortality ca. 20 percentage points higher than predicted by independent action). Similar levels of synergism were observed under the markedly different conditions of the field and greenhouse environments, and the synergism was confirmed as arising from interaction of the two micobes, as the Bt spray carrier had no significant effect on efficacy of the Mycotrol product and the Bb spray carrier had no effect on the efficacy of Novodor. The great capacity of these two control agents to act in concert to control CPB is well documented (the fast-acting, toxic Bt acting to protect potato crops from defoliation and the slow-acting Bb reducing survival to the adult stage). These finding further underscore the strong complementary action of these agents applied jointly against CPB.

Key words: Bacillus thuringiensis morrisoni; *Beauveria bassiana*; *Leptinotarsa decemlineata*; Interaction; Synergism

1. Introduction

In a previous study (Wraight and Ramos, 2005) we reported observations of a synergistic interaction between the common fungal pathogen *Beauveria bassiana* (strain GHA formulated as Mycotrol® ES) and a coleopteran-active subspecies (serovariety) *of Bacillus thuringiensis*, *B. thuringiensis tenebrionis* (formulated as Novodor® FC) applied jointly (as tank-mixes) against larval populations of Colorado potato beetle *Leptinotarsa decemlineata* (CPB). This pathogen is currently considered a strain of *B. thuringiensis morrisoni* (Reyes-Ramirez and Ibarra, 2005). In that study, the mechanism underlying the synergistic activity was left to conjecture. Indeed, from

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the experiments conducted, it was not discernible if the observed response derived from direct interaction between the pathogens or indirectly from interactions between one pathogens and the "inert" ingredients comprising the base formulation or carrier of the other pathogen or even from interactions between the two formulation carriers. As the tests reported by Wraight and Ramos (2005) were conducted under open-field conditions, involvement of other natural enemies of the Colorado potato beetle also could not be ruled out.

Since our initial investigations, additional studies of the interactions between entomopathogenic fungi and Colorado potato beetle have been reported. Kryukov et al. (2008) conducted a series of experiments with essentially unformulated aqueous suspensions of conidia of *Metarhizium anisopliae* (Ma) and spores and crystalline inclusion bodies (crystals) of *B. thuringiensis* strain 2495/N8ab (identified as B. thuringiensis ssp. *morrisoni* var. tenebrionis). Under laboratory and field conditions (larvae caged on potato foliage), it was found that mixtures of these pathogens acted synergistically to both accelerate and increase larval mortality. It was concluded that feeding disruption resulting from Bt toxinosis was the most likely cause of the enhanced mortality, impacting host physiology, impeding growth, lengthening the intermolt period, and interfering with the molt, all factors that have been shown to affect susceptibility of insects to fungal infection (Vey and Fargues, 1977; Vandenberg et al., 1998; Furlong and Groden, 2003; Ugine et al, 2005). However, findings from the Kryukov et al. (2008) studies and those by Wraight and Ramos (2005) could not rule out the possibility that fungal infection increased larval susceptibility to *B. thuringiensis*. Kryukov et al. (2008) reported that "analogous results were obtained in laboratory experiments with mono- and mixed infections of Colorado potato beetle larvae with *Beauveria bassiana* and *B. thuringiensis*," but no experimental details or data were reported.

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The objective of the present study was to investigate potential effects of commercial formulation carriers on the *B. bassiana-B.thuringiensis* interaction and to provide further insight into the nature of the interaction via observations of treated CPB larvae on uncaged potato plants under conditions of variable temperature and relative humidity.

2. Materials and methods

Studies were conducted in a research greenhouse located at the R. W. Holley Center for Agriculture and Health, Ithaca, New York, and in field plots on Cornell University's H. C. Thompson Vegetable Crops Research Farm in Freeville, New York.

2.1. Biopesticide preparations

A commercial paraffinic-oil-based formulation of *B. bassiana* strain GHA (Mycotrol ES) containing 2.1 x 10^{10} conidia/ml and a flowable concentrate of *B. thuringiensis tenebrionis* (Novodor FC) containing 17,224 *Leptinotarsa* units/ml were used in the greenhouse experiments. The Novodor FC formulation and a clay-based wettable powder formulation of *B. bassiana* (Mycotrol WP) containing 4.4 x 10^{10} conidia/g were used in the field tests. Batches of Mycotrol were stored at 4ºC, and batches of Novodor were stored at room temperature in an air-conditioned pesticide storage facility. Prior to each test, viability of *B. bassiana* conidia was determined as described by Wraight et al. (2005). In all cases, viability was high (> 90% germination within 16–18 h incubation on 0.5% yeast extract agar). Doses were adjusted by the estimated rates of viability.

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2.2. Greenhouse tests

2.2.1. Insects and plants

CPB eggs were obtained from a laboratory colony maintained at the Phillip Alampi Beneficial Insects Laboratory of the New Jersy Department of Agriculture in Trenton, NJ. The colony had been in maintenance on potato for > 20 years. Eggs were incubated at 25ºC in Petri dishes (15-cm diam.) provisioned with potato foliage (ca. 200 eggs per dish). After molting to the second instar, groups of 15 or 20 larvae were transferred to small Petri dishes lined with wet filter paper. The groups were then assigned at random to the spray treatments.

Potato tubers ('Atlantic') were cut into seed pieces and planted individually in 15-cm-diam. pots containing Metro-Mix 360 (Scotts, Marysville, OH) and maintained in the greenhouse. Sprouts were pruned to leave a single stem in each pot. Plants were maintained in the greenhouse for 2–3 week, reaching 15–25 cm in height, before being randomly assigned to the experimental treatments.

2.2.2. Biopesticide spray suspensions

Spray suspensions were prepared by mixing either Mycotrol ES or Novodor FC or the two products together in distilled water with no added surfactants. For principal investigations of synergism, the two biopesticides were applied at their approximate LC_{50} doses. Preliminary greenhouse tests with second-instar CPB larvae identified these rates as 0.0094% Mycotrol and 0.017% Novodor (47 µl and 85 µl were mixed in 500 ml DH_20, corresponding to concentrations of ca. 2 x 10^6 conidia/ml and 2.9 *Leptinotarsa* units per ml). Treatments also included the above-

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described dose of Mycotrol in combination with a sublethal dose of Novodor (0.0005%). Within-test treatments and treatment combinations are summarized in Table 1. Henceforth, the Mycotrol and Novodor treatments will be identified as Bb and Bt and the mixture as Bb + Bt. The low Bt dose will be identified as Bt-L (Bt-low)

2.2.3. Test protocols

Fifteen or 20 larvae from one of the above-described batches were transferred to the exposed dorsal (adaxial) surfaces of leaves near the top of a potato plant (dispersed as evenly as possible across the primary, fully-expanded leaves), and the spray treatment was immediately applied. Infested plants were sprayed individually in a Burgerjon spray tower (Burgerjon 1956) fitted with an air-atomizing nozzle (Fluid Cap 2850 + Air Cap 70) retained in a 1/4 J Nozzle body (Spraying Systems, Wheaton, IL); the nozzle was provided with a constant airflow of 10 L/min. Spray deposition at a level corresponding to the top of the plant was ca. 0.01 µl/mm^2 (resulting from spraying a 5-ml aliquot). The plant was situated on a rotating turntable (33 rpm) during application. Conidial deposits were sampled by pinning plastic cover slips to the upper and lower surfaces of potato leaves near the top center of the plants (two cover slips per plant), and these deposits were quantified using the protocol described by Wraight et al. (1998). Each treatment was applied to five or six replicate plants.

Five tests were conducted on different occasions using different generations of beetle larvae. Each set of treatments in each test included a spray control, Bb alone, Bt alone, and the Bb + Bt mixture (Table 1). The Bb-ES spray control (formulation carrier ingredients alone, without conidia, at 0.0094%) was used for the spray control in all tests; preliminary greenhouse

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tests indicated no differences between blank treatments and untreated controls. The 14 and 28 March tests included independent sets of treatments with the sublethal dose of Bt.

Following treatment, each plant was placed uncaged in the center of a large plastic tray (54 cm x 42 cm x 6.5 cm deep) on a greenhouse bench. The plants were arranged in a randomized complete block design: five or six blocks with one replicate of each treatment per block. The experiment was blocked across the length of the North/South-oriented greenhouse (blocks one and five located at opposite ends of the greenhouse). The plants were incubated under natural light conditions; however, daily photoperiod was effectively maintained at ca. 14 h by stray light from adjacent greenhouses. Each day, plants were watered as needed, dead larvae were removed, and any live, fallen larvae were returned to the plant. The larvae were easily contained, being unable to climb the smooth walls of the trays. During tests 1–4, mortality of larvae was recorded daily. After 7 or 8 days, the plants and pots were carefully examined, and total numbers of dead and surviving larvae were tallied.

Greenhouse temperature and relative humidity (RH) were recorded hourly by Hobo electronic data loggers (Onset Computer, Bourne, MA). During tests conducted under spring and fall months (late March, April, and early November), the greenhouse was not heated. Overnight temperatures generally dropped to 10–15ºC, and the evaporative-cooling system was activated during days when temperatures rose above 25ºC. During warm days with high insolation, the cooling system was unable to hold temperatures below 30ºC. During cold months (mid-November–December), the greenhouse was heated, with controls set to maintain temperature at ca. 25ºC. Environmental conditions during each test are summarized in Table 2.

2.3. Field tests

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2.3.1. Research plots

Two field tests were conducted during the 2005 and 2006 field seasons using essentially the same protocols reported by Wraight and Ramos (2015). The test site was established to encompass a balanced incomplete block design comparing nine treatments in 12 blocks of three experimental units (plots) with four replications (Cochran and Cox, 1957). Potato plants, rows, plots, and blocks were established as described by Wraight and Ramos (2015), except that each 68-m-long block containing six plots was divided into two blocks of 34 m, each with three plots. Six of these 68-m sets of rows were established to accommodate the total of 12 blocks and 36 plots, each plot measuring 6 rows by 9.1 m. Plots within each 68-m set of rows were separated by 2.5 m bare ground, and a 3.4-m corridor of bare ground separated each of the blocks.

2.3.1. Spray applications

The above-described Novodor FC and Mycotrol WP commercial products were used in both field tests. The nine treatments and treatment combinations with their codes/abbreviations are summarized in Table 1. The sprayer was configured as described by Wraight and Ramos (2015) and operated at the "low pressure" of 345 kPa, delivering a volume of ca. 480 L/ha.

In 2005, potatoes ('Keuka Gold') were planted 18 May, and treatments were applied 28 June and 2 July. In 2006, potatoes (also 'Keuka Gold') were planted 9 May, and treatments were applied 29 June and 5 July. As for the greenhouse tests, the Novodor and Mycotrol treatments will henceforth be referred to as Bt and Bb and the mixture as Bt + Bb. In our previous study of Bt x Bb interactions, results from an experiment comparing the Mycotrol ES and WP formulation, results were very similar (Wraight and Ramos, 2005). Chemical pesticides were

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applied prophylactically each year for late-blight management, as described by Wraight and Ramos (2005). Fungicides (primarily maneb, mancozeb and chlorothalonil were applied at 7–10 day intervals in rotation; however, in the field tests reported here, initiation of the spray programs was delayed until at least 2 days after the last biopesticide application. Insecticides for leafhopper control were not applied until August.

2.3.2. Sampling protocols

Protocols for CPB population sampling, sampling of Bb spray deposits (conidia) on potato foliage, and assessment of crop damage (defoliation) were the same as described by Wraight and Ramos (2015). In 2005, larval populations were sampled 28 June and 5, 8, 11, and 13 July; defoliation damage was assessed on 18 July. In 2006, larvae were sampled 28 June and 5, 6, 7, 11, 13, and 14 July. Defoliation was assessed on 26 July and first-generation adults were sampled 24 and 27 July. All samples of CPB larvae collected on the day of a spray treatment were collected prior to the sprays. Weather data from the 2005 and 2006 field seasons are presented in Wraight and Ramos (2005).

2.4 Statistical analyses

Mixed-model ANOVAs were conducted using JMP Pro version 12.2.0 (SAS Institute, Cary NC). In the analyses of greenhouse-test results, percent mortality data (binomial proportions) were transformed to empirical logits and weighted by inverse-variance prior to ANOVA (see Jaeger 2008). Tests (different dates) and experimental blocks were treated as random effects. Fixed effects included nominal variables representing application vs. no application of each

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biopesticide (Bt or Bb) and the Bt x Bb interaction. The spray control (Bb carrier) represented the no Bt/no Bb treatment in the interaction analysis. In the initial analyses, random effect interactions were included in the models; however, in all cases the main effect block and all block interactions collectively accounted for < 4% of total variance, and block was removed from further analysis to provide additional degrees of freedom for testing significance of the fixed effects and their interaction.

In the analyses of field-test results, numbers of larvae were log-transformed prior to ANOVA. Analyses were conducted on means derived from total numbers of CPB larvae in multiple samples collected over specified periods of time, precluding complications associated with repeated measures. From the 2005 data, numbers of total larvae were determined from samples collected 7, 10, 13, and 15 days after the initial spray on 28 June; from the 2006 data, totals were from samples collected 8, 12, 14, and 15 days post-initial spray on 29 June. Models included year and field block as random effects; fixed effects were as described above, but included application vs. no application of the formulation carriers. Interaction terms included Bt x Bb carrier, Bb x Bt carrier, Bt carrier x Bb carrier, and Bt x Bb. Defoliation percentages (non-binomial proportions) were transformed to standard logits prior to ANOVA. The highest proportion defoliation ($p = 0.95$) recorded in a replicate plot was subtracted from 1, and this value (0.05) was added to the numerator and denominator in the logit calculation, $\ln[(p + 0.05)/(1 – p) + 0.05]$, to enable inclusion of 100% values (Warton and Hui, 2011). This transformation, however, did not resolve the problem of unequal variances among the means from the 2006 test, and results were compared to those from an ANOVA of the rank-transformed data as recommended by Conover (1999).

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3. Results

3.1. Greenhouse tests

Responses of CPB to Bt and Bb were highly variable across the tests conducted under differing environmental conditions (Table 3). This variability was reflected in the initial overall analysis, with the Test x Bt and Test x Bb interactions accounting for 26% and 29% of total variance, respectively. These random-effect interaction terms were retained in the model. In contrast, the Bt x Bb interaction was consistent across tests, with the Test x Bt x Bb interaction accounting for just 3% of total variance; this interaction term was removed from the model. Results are presented in Table 4. The Bt and Bb treatments applied independently at the 0.017 and 0.0094% rates produced 47 and 34% control-corrected mortality, respectively. Mortality predicted by independent action (combination of independent probabilities) was 65%. Mortality observed in the combination treatment was 23 percentage points higher (88%), and this positive interaction (synergism) was statistically significant (P = 0.008). The estimated increase was even greater (34%) based on the back-transformed LS means. In contrast, no significant interaction was observed when the same rate of Bb was combined with the low (sublethal) dose of Bt (P = 0.47).

Mean numbers of conidia in spray deposits were 33% greater on plants sprayed with Bb compared to plants sprayed with the Bb-Bt mixture (65 ± 9.5 vs. 49 ± 6.6 viable conidia/mm^2), even though an equal amount of Bb was added to each preparation. This difference, however was not significant ($F_{1,3.6}$ = 1.0, P = 0.37). In this analysis, the random effects Test and the Test x Treatment interaction contributed to 22 and 25% of total variance, respectively. Deposition

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appeared to be most affected by height of the potted potato plants in the spray tower, but this variable was not measured across tests. The Burgerjon spray tower generates a fine mist that settles onto the target, and lower leaf surfaces received < 3% of total conidia.

The data were analyzed further by inclusion of greenhouse environmental conditions in the model. Conditions were characterized as unheated (temperature fluctuating greatly between day and night hours vs. heated (temperature held relatively constant) (Table 2). The fixed effect Heated in the model (Table 5) was evaluated as a categorical variable (with vs. without heating). Interactions involving the random effect Test contributed little to overall variability: all contributions were near zero, except for minor contributions from the 3-way interactions Test x Bb x Heated (13.8%) and Test x Bt x Bb (3.5%). These terms were ultimately excluded from the analysis, retaining test only as a main blocking effect. Greenhouse temperature had a nearly opposite effect on the two pathogens. Heating reduced Bb-induced mortality from 62 to 22% but increased Bt-induced mortality from 28 to 69% (Table 5). Simple regressions of percent mortality on mean temperature revealed a strong, direct relationship between temperature and mortality from the Bt treatments ($R^2 = 0.566$) and an equally strong, indirect relationship between temperature and mortality from the Bb treatments ($R^2 = 0.578$) (Table 3). These results are reflected in interaction tests reported in Table 5. Both the Heated x Bt and Heated x Bb interactions were highly significant, whereas the 3-way interaction Heated x Bt x Bb was clearly insignificant ($P = 0.48$), and the levels of synergism were similar under the heated vs. unheated conditions (+22 vs. +18 percentage points).

Daily observations revealed significant mortality of larvae beginning on days five and six in the Bt and Bb treatments, respectively, under heated greenhouse conditions (Fig. 1). Under cooler (unheated) conditions, substantial mortality did not occur until 1–2 days later. Response

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of beetles to the combination treatment differed little from the response to Bt alone, with onset of significant mortality occurring on day five under heated greenhouse conditions and on day 6 under cooler conditions.

3.2 Field tests

Results from sampling of the CPB larval populations before and after application of the various spray treatments in 2005 and 2006 are presented in Fig. 2, and analyses of the data are shown in Tables 6 and 7. Graphic presentations of pest population trends were simplified by combination (pooling) of the untreated and formulation-carrier controls, which did not differ significantly ($P = 0.56$ in both experiments). Bb alone provided no control of larvae in 2005 (larval populations were actually larger in the control vs. Bb-treated plots), and only 30% reductions were recorded in 2006. In contrast, Bt consistently reduced CPB numbers by > 50%, and the combination of Bt and Bb provided ca. 80–85% control. Levels of synergism, indicated by significant Bt x Bb interactions (P values ≤ 0.004) were +20 to +23 percentage points, similar to the levels observed in the greenhouse tests and in our earlier tests (Wraight and Ramos 2005).

The present studies were expanded to investigate potential effects of the formulation carriers. In both 2005 and 2006, neither spray carrier (Bt C nor Bb C) had any significant impact on larval populations when applied alone or in combination (all main effect P values ≥ 0.16 and Bt C x Bb C interaction P values ≥ 0.26) (Tables 6 and 7). Activity of each biopesticide was also unaffected by combination with the carrier of the other biopesticide (Bt x Bb C and Bb x Bt C interaction P values ≥ 0.35). Combined results from the 2005 and 2006 studies are presented in Fig 3).

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In accord with our findings from parallel studies conducted in 2006 (Wraight and Ramos, 2015), greatest expression of *Beauveria* efficacy occurred after the larvae entered the soil to pupate, ultimately resulting in greatly reduced emergence of next-generation adults (Fig. 2, Table 8). Sampling of the adult populations was initiated too late to identify peak emergence in 2006, as adults began to emerge earlier than anticipated. It is also evident from the data (Fig. 2) that adults were migrating from heavily damaged control plots into treatment plots, which is reflected in the decrease vs. increase in beetle numbers in the control vs. treatment plots between 24 and 27 July (in 2005, damage was so severe in controls and migrations so extensive that adult sampling was not attempted). Data presented in Table 8 are thus based solely on the 24 July samples. To a large extent, efficacy of the Bt treatments was apparently also delayed, with mortality continuing to occur after termination of larval sampling; estimated control of CPB larvae by Bt alone was 51%, but emergent-adult populations were reduced by > 95% (Tables 7 and 8). With regard to the larval populations, control of adults was greatest in the Bt + Bb treatment, but only by a few percentage points, and ultimately, the observed level of control afforded by the combination treatment was ca. two percentage points lower than the expected value. This negative interaction was consistent across replicates and statistically significant ($P < 0.0001$), but of no significance in the context of microbial biocontrol, as all treatments achieved > 95% control of the summer adult population.

The lack of larval control by Bb in 2005 vs. the low level of control (30%) achieved in 2006 (Fig. 2) are even more sharply contrasted in the observed rates of defoliation (Fig. 4). In terms of percent reduction in damage compared to the controls, Bb reduced defoliation by 81% in 2006, but by < 1% in 2005. Greatest crop protection was afforded by the combination treatments in both years; however, the greater than predicted level of protection in 2005 (+12.1

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percentage points) and the less than predicted level in 2006 (– 4.5 percentage points) were not statistically significant (Bt x Bb interaction $F_{1,24.1} = 1.3$, $P = 0.27$ and $F_{1,25.6} = 2.6$, $P = 0.12$, respectively).

Spray deposition samples collected from the dorsal vs. ventral surfaces of six plants in each of the Bb, Bb C, and Bt + Bb treatments plots (1 coverslips/surface/plant) on each of the two spray dates in the 2005 field test revealed mean (± standard error) depositions of 812 ± 84 vs. 165 ± 45, 813 ± 84 vs. 165 ± 45, and 783 ± 85 vs. 157 ± 61 conidia/mm^2, respectively. Depostion was clearly unaffected by addition of Bt or Bt C to the Bb formulation. Only 14% of total conidia were deposited on the lower leaf-surfaces. Sampling of conidial deposits in multiple Bb-treatments plots was not conducted in 2006; however, the study by Wraight and Ramos (2015) includes data from the Bb-alone treatment (the UTC, Bb C, and Bb treatment plots included soil cages, as part of the study of delayed effects of *B. bassiana* treatments).

4. Discussion

These investigations revealed that the synergistic interaction between *B. thuringiensis tenebrionis* and *B. bassiana* applied against larval populations of Colorado potato beetles was remarkably consistent over a great range of conditions. The level of synergism (mortality ca. 20 percentage points higher than predicted by independent action) was observed under both warm and cool conditions in a very dry greenhouse and under wet field conditions. It is well documented that these pathogens are most virulent under contrasting condition. *B. bassiana*, like most entomopathogenic fungi, is most infectious under cool–moderate temperature and high humidity conditions, at least with respect to the microclimate at the site of infection (see Jaronski,

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2010) and *B. thuringiensis*, like most toxin-based insecticides, is most virulent under warm conditions, with humidity having less significant, less predictable effects (see Glare and O'Callaghan, 2000). Under the highly variable conditions of these studies, low activity of one pathogen was counterbalanced by high activity of the other pathogen, with the result being a consistent level of synergism. Formulation carriers did not affect the interaction observed between the two biopesticides.

As emphasized in our previous study (Wraight and Ramos, 2005), the levels of synergism recorded, although consistent, are not high. It is thus not surprising that statistically significant Bb x Bt interaction is undetectable by the time the larvae reach the adult stage. In tests of second-instar CPB, Kryukov et al. (2009) also reported levels of mortality from exposure to *M. anisopliae* alone becoming nearly equal to those from *M. anisopliae* mixed with *B. thuringiensis* within 8 days post-treatment. These observations, combined with other results, strongly suggest that the synergism derives from the bacterial toxinosis leaving the CPB larvae more susceptible to infection by the less virulent, slow-acting fungal pathogen. If the basis of the underlying mechanism was a temporary increase in food consumption induced by *B. bassiana* infection (Fargues et al. 1994), leading to greater initial intake of toxin, one would expect to see reduced survival times for larvae exposed to the combination treatment, particularly under warm conditions on plants protected from UV radiation (protection afforded by the double-pane polycarbonate glazing of the greenhouse). However, this was not the observed result (Fig. 1).

The specific mechanism(s) underlying the synergism remain unknown but undoubtedly involve a combination of effects on host physiology described by Vey and Fargues (1977) and Furlong and Groden (2003) and hypothesized by Wraight and Ramos (2005) and Kryukov et al. (2009). The bacterial toxinosis interrupts feeding by CPB larvae. This leads to delayed molting,

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providing a longer window of time for fungal infection, and starvation stress that likely compromises the larval immune response.

The relatively slow speed of kill exhibited by *B. thuringiensis* reported here was unexpected. Kryukov et al. (2009) reported much more rapid mortality of second-instar larvae, with the fungus-alone (*M. anisopliae*) producing 40–60% mortality and the combination treatment producing 80–90% mortality within 4 days in laboratory tests and within 5 days in the field. Comparisons are difficult, however, as the concentration of *B. thuringiensis* in the Kryukov et al. (2009) study was expressed only in terms of spores/ml, larvae and potato foliage were dip-inoculated with the pathogens, and test temperature and relative humidity conditions were not reported. In addition, although it was claimed that similar results were observed in tests of *B. bassiana*, results reported were only those from tests of *M. anisopliae*. Ferro and Gelernter (1989) reported on the CPB-larvicidal activity of a new variety of *B. thuringiensis* identified as san diego that was later described as indistinguishable from strain tenebrionis (Krieg et al., 1987; Reyes-Ramirez and Ibarra, 2005). From laboratory bioassays against second-instar larvae conducted at 25ºC, Ferro and Gelernter (1998) determined a median lethal concentration of 0.0204 mg *B. thuringiensis*/ml (0.204 mg of a 10% technical powder/ml) and median survival time of 76 h. Based on information from the Novodor FC material safety data sheet, the formulation had a bulk density of ca. 1.1 (1.05–1.15) g/ml and contained 10% *B. thuringiensis* (3% toxin). Our concentration of 0.017% translates to a comparable rate 0.0187 mg *B. thuringiensis*/ml. It is likely, however, that actual doses consumed were substantially greater in the Ferro and Gelernter (1989) tests, as the larvae were confined on dip-inoculated potato leaflets. In our tests, larvae on potato plants were targeted with a low-volume spray (substantially below runoff) that produced minimal deposition of material on leaf undersides and other

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unexposed surfaces. The rate of Bt applied in our field tests (2.34 L Novodor in a volume of 480 L water/ha) translates to a concentration of ca. 5.4 mg/ml. This dose, combined with Bb reduced larval populations by 60% within 7 days in 2005 and by 62% within 6 days in 2006, much slower than the above-described activity reported by Kryukov et al. (2005) from their field tests of *M. anisopliae*.

Mortality due to *B. bassiana* mycosis was nearly 3-fold greater (62 vs. 22%) under unheated- vs. heated-greenhouse conditions (Table 5). This is initially surprising, considering that humidity levels were only slightly higher in the unheated greenhouses. However this finding underscores the great influence of temperature on *B. bassiana* activity against CPB. Warmer conditions accelerate larval development, reducing inter-molt periods, and though conidia were protected from UV degradation in the greenhouse, it has been demonstrated that acquisition of conidia from a treated substrate by CPB larvae (e.g., following a molt) is much less likely to yield a lethal dose than direct, topical-spray application (Fernandez et al., 2001). It is also well documented that the GHA strain of *B. bassiana* used in these tests is highly sensitive to temperatures ≥ 30ºC (Long et al., 2000; Martin et al., 2000; Inglis et al., 1996). The lowest mortality from Bb (16%) was recorded under the highest temperature conditions (mean 29.7ºC in Test 5). There was a near-perfect correlation between temperature and RH in the five greenhouse tests ($r = -0.946$).

The complete inability of the 2005 Bb-alone treatments to provide any protection from crop damage (Fig. 4) can be explained, at least in part, by the overwhelming pest populations that developed in 2005, particularly in the Bb test plots (Fig. 4). In contrast, the 2006 Bb treatments reduced defoliation by > 80% (from 65% to 12% damage). This was a much greater level of crop protection than observed in our previous studies, and is apparently attributable to highly

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favorable conditions for fungal activity in 2006. Cool, wet conditions prevailed for much of the test, including the first few days following each application (see Wraight and Ramos, 2015). The fact that an 80% reduction in damage was achieved even though larval populations were reduced by just 32%, supports the observations of Fargues et al. (1994), who noted dose-dependent 57–76% reductions in feeding by *B. bassiana*-infected larvae following a brief period of increased consumption.

This study confirms our previous findings of a consistent synergistic interaction between the biopesticides Novodor and Mycotrol and reveals that the interaction can indeed be attributed to actions of the two active ingredients, *B. thuringiensis morrisoni* strain tenebrionis and *B. bassiana* strain GHA. Also confirmed, however, is the fact that the synergism is of only a low level. This supports the conclusion by Wraight and Ramos (2005) that the synergism exhibited by the Bt-Bb combination is less important in the context of microbial biocontrol than the high levels of compatibility and complementarity of the two control agents. Results from the greenhouse study reveal yet another aspect of the complementarity, i.e., the capacity of the pathogen mixture to act under a broader range of conditions than either agent alone. Similar complementary action has been shown with mixtures of *Metarhizium acridum* with *B. bassiana*, in those cases *M. acridum* (formerly identified as *M. flavoviride*) being active at higher temperatures than *B. bassiana* (Inglis et al. 1997).

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and pest and disease control. This paper reports research only. Mention of a proprietary product does not constitute an endorsement or recommendation for its use by the USDA.

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Figure legends

Fig. 1. Daily mortality of Colorado potato beetle larvae treated with Bb, Bt, or a mixture of Bb and Bt in four greenhouse tests: two tests conducted under heated conditions and two under unheated conditions. Mortality is expressed as daily percent of total mortality recorded during each test (mean ± standard error). Treatment codes are explained in Table 1.

Fig. 2. Trends in larval and first-generation adult Colorado potato beetle populations treated with Bb, Bt, or a mixture of Bb and Bt during two field tests. Means are larvae per 10 stems per replicate field plot per sample date (samples collected 7, 10, 13, and 15 days after initial application in 2005 and 8, 12, 14, and 15 days after initial application in 2006). Vertical lines represent standard errors of means (n = 4); for clear presentation of standard errors, some means are offset on the x-axis. Treatment codes are explained in Table 1; data identified as "controls" represent the means of the four treatments without active ingredients (UT, Bb C, Bt C, and Bb C + Bt C), which did not differ significantly.

Fig. 3. Combined results from the 2005 and 2006 field tests. Mean larvae per 10 stems per replicate field plot per sample date from four consecutive samples collected 7, 10, 13, and 15 days after initial application in 2005 and 8, 12, 14, and 15 days after initial application in 2006. Treatment codes are explained in Table 1. Vertical lines represent standard errors. Means with same letter are not significantly different (Tukey test, log-transformed data, alpha = 0.05).

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Fig. 4. Percent defoliation in 2005 and 2006 field-test plots. Defoliation estimated from one randomly selected plant from each outside row and two from each interior row of each 6-row test plot (total of 10 plants per plot, 40 plants total from four replicate plots per treatment). Vertical lines represent standard errors. Means within years with same letter are not significantly different (Tukey test, 2005: log-transformed data, 2006: rank-transformed data, alpha = 0.05).

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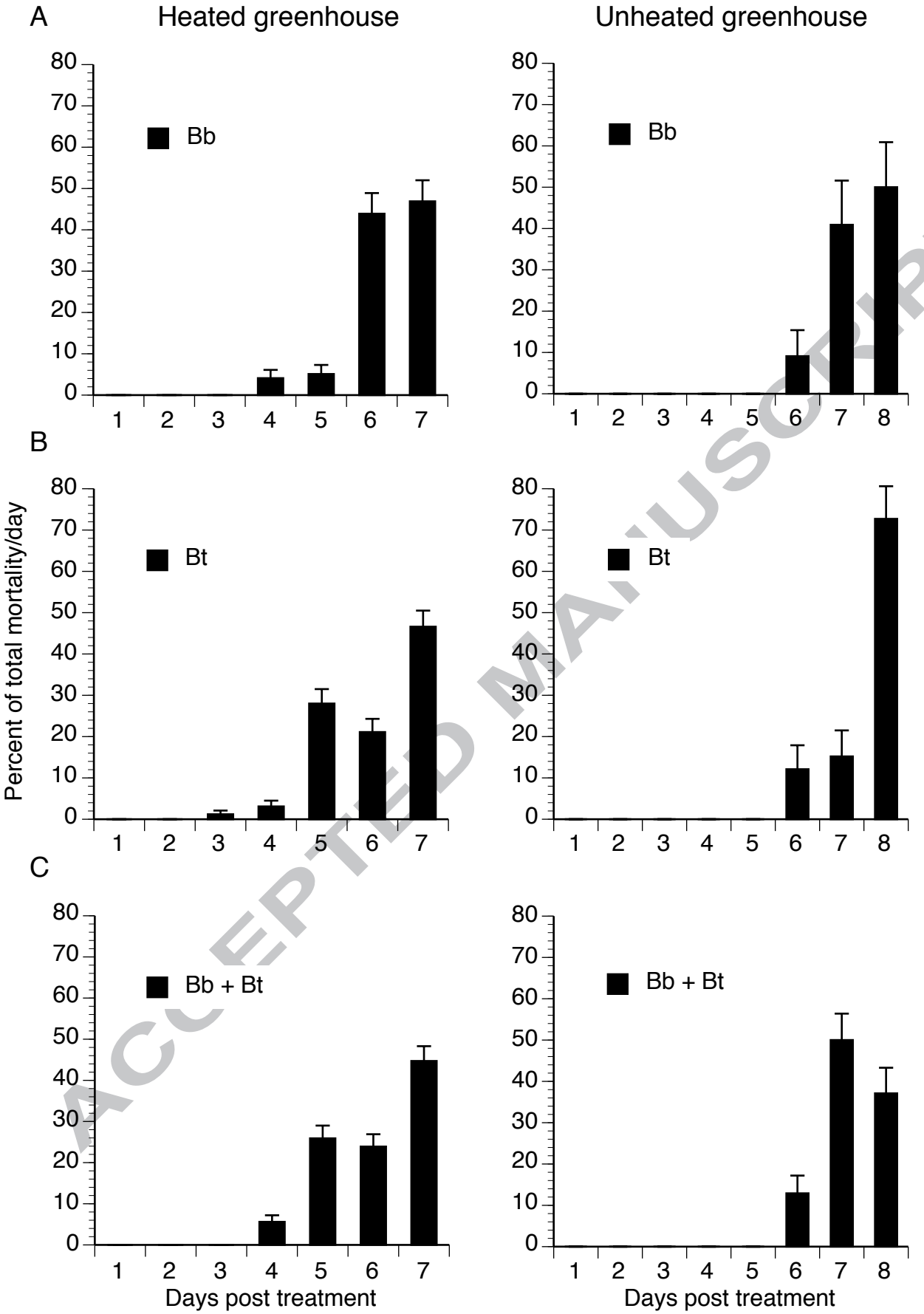

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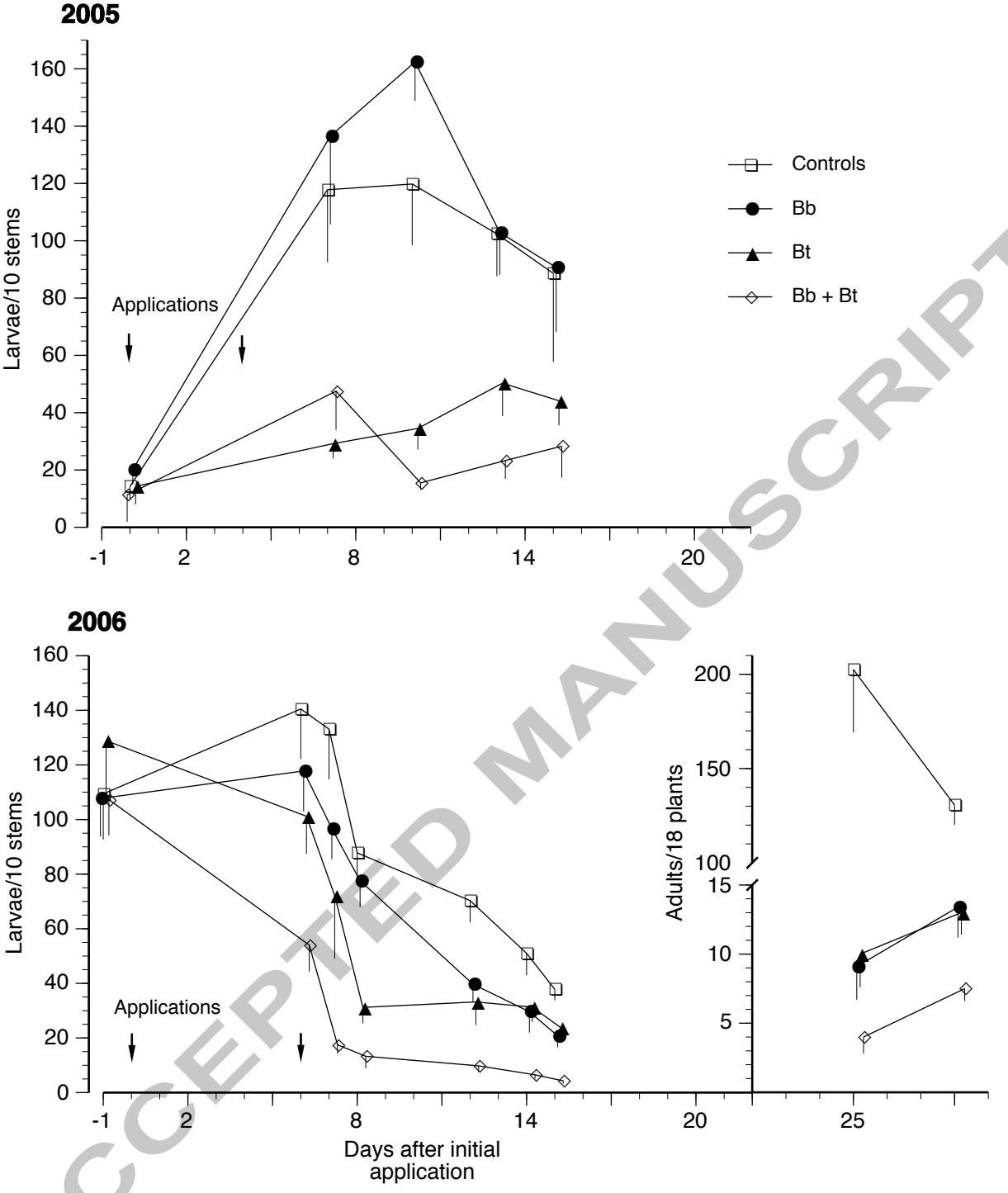

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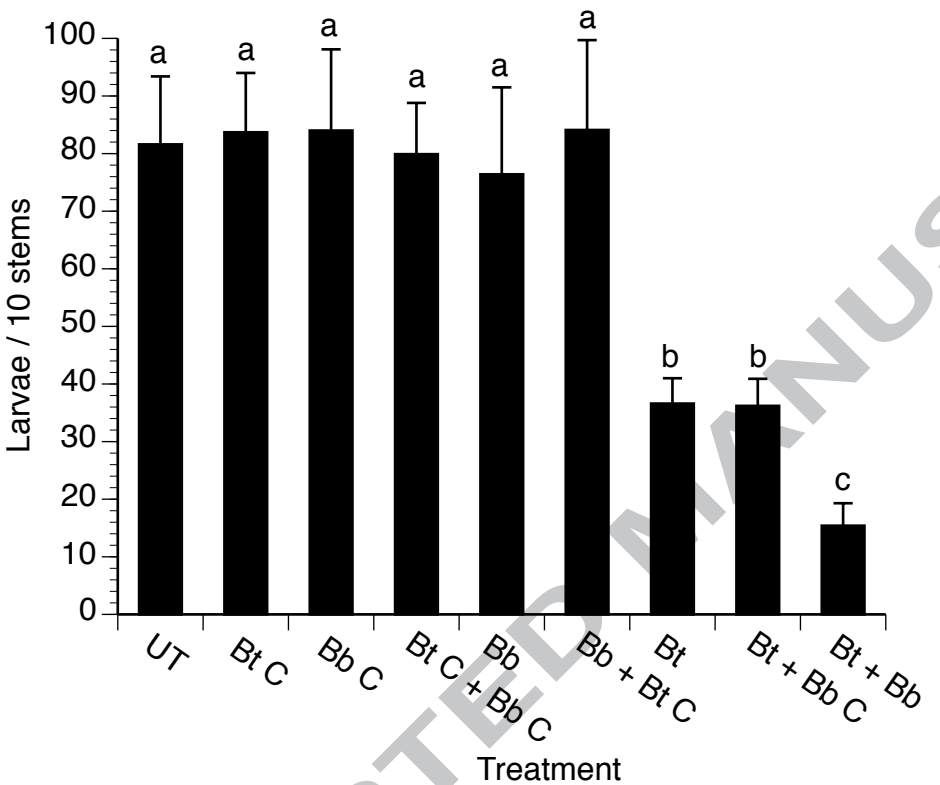

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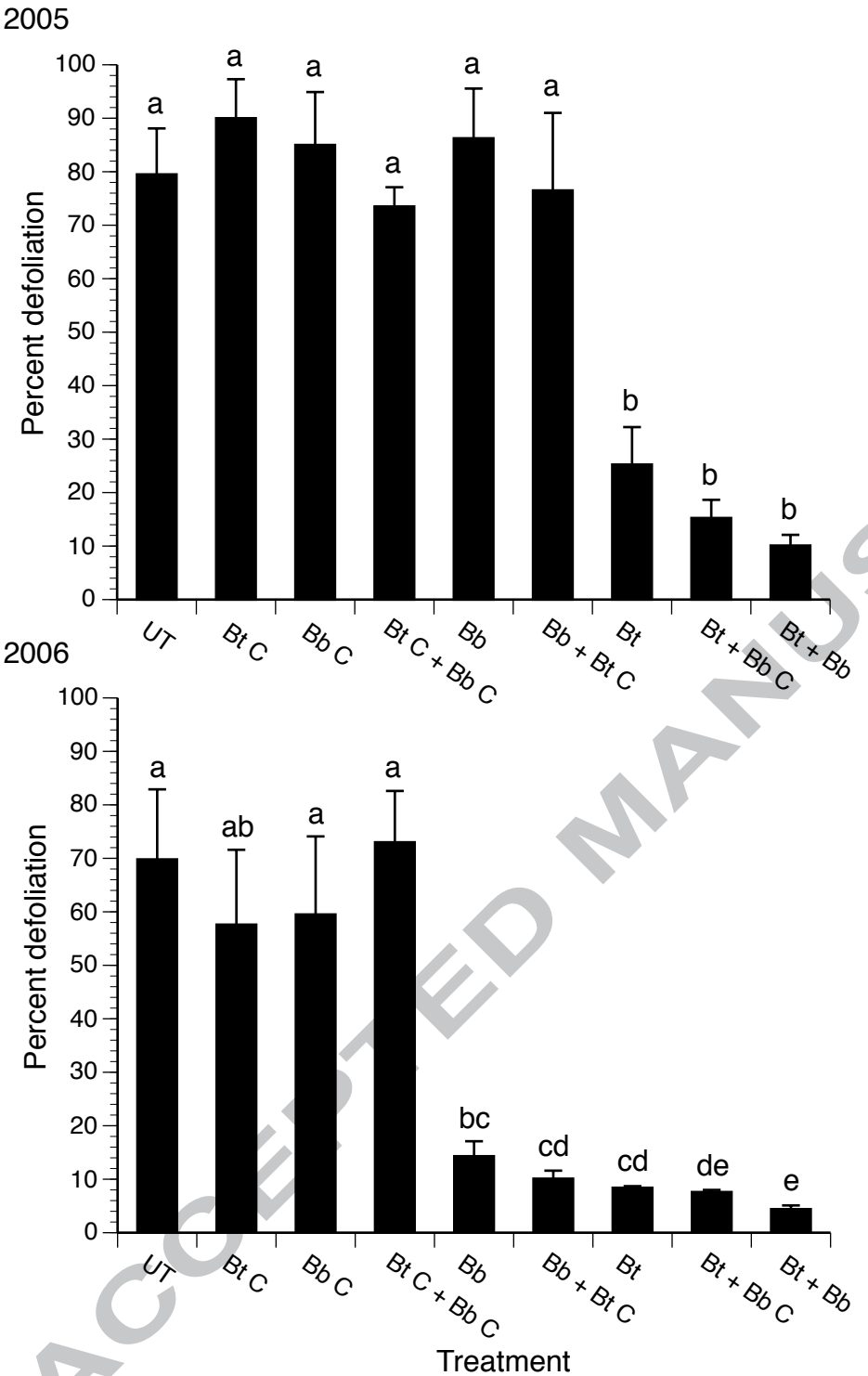

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Table 1. *Bacillus thuringiensis morrisoni* strain tenebrionis (Novodor®) and *Beauveria bassiana* strain GHA (Mycotrol®) treatments applied in field and greenhouse tests.

Treatments	Code
Greenhouse tests using Novodor FC and Mycotrol ES formulations (2000–2001)	
Spray control: Mycotrol carrier at 0.0094% [a]	Bb C
Novodor FC at 0.017%	Bt
Mycotrol ES at 0.0094%	Bb
Novodor FC at 0.017% mixed with Mycotrol ES at 0.0094%	Bt + Bb
Spray control: Mycotrol ES carrier at 0.0094%	B C
Novodor FC at 0.0005%	Bt-L
Mycotrol ES at 0.0094%	Bb
Novodor FC at 0.0005% mixed with Myctotrol ES at 0.0094%	Bt-L + Bb
Field tests using Novodor FC and Mycotrol WP formulations (2005–2006)	
Controls	
Untreated	UT
Novodor carrier at 2.34 L/ha [b]	Bt C
Mycotrol carrier at 0.56 kg/ha [c]	Bb C
Novodor carrier mixed with Mycotrol carrier (at above-indicated field rates)	Bt C + Bb C
Treatments	
Novodor at 2.34 L (40.3 x 106 LTU)/ha	Bt
Mycotrol at 0.56 kg (2.5 x 10^{13} conidia)/ha	Bb
Novodor mixed with the Mycotrol carrier (at above-indicated field rates)	Bt + Bb C
Mycotrol mixed with the Novodor carrier (at above-indicated field rates)	Bb + Bt C
Novodor mixed with Mycotrol (at above-indicated field rates)	Bt + Bb

[a] Mycotrol ES formulation blank provided by manufacturer.

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[b] Heat-treated product (containing heat-killed spores and denatured crystal toxin); application rate corresponding to the product low label rate of 2.34 L/ha (1 quart/acre).
[c] Mycotrol WP formulation blank provided by manufacturer; application rate corresponding to the product medium label rate of 0.56 kg/ha (0.5 lb/acre).

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Table 2. Environmental conditions during five greenhouse tests of *B. thuringiensis* and *B. bassiana* against Colorado potato beetle described in Table 1.

Test	Greenhouse [a]	Test date (number of days)	Temperature (ºC) hourly mean (range) [mean daily min/max]	Relative humidity (%) Hourly mean (range) [mean daily min/max]
Test 1	Heated	15–22 Nov 2000 (7)	26.3 (24.4–28) [25.4.6/27.4]	22 (15–30) [18/22]
Test 2	Heated	6–13 Dec 2000 (7)	26.2 (24.4–27.5) [24/27.1]	22 (11–39) [16/28]
Test 3	Unheated	14–22 Mar 2000 (8)	21.3 (15.2–38.8) [15.9/30.4]	28 (22–43) [24/37]
Test 4	Unheated	28 Mar–5 Apr 2001 (8)	17.6 (14.5–25.2) [15.7/20.4]	38 (12–65) [28/48]
Test 5	Heated	30 Oct–6 Nov 2001 (7)	29.7 (20.2–32.8) [25.1/31.4]	21 (14–40) [17/30]

[a] Greenhouse heated to maintain warm-temperature, low humidity conditions vs. unheated, allowing temperature and humidity to fluctuate with the solar cycle.

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Table 3. Percent mortality (± standard error) observed in five greenhouse tests of *B. thuringiensis* and *B. bassiana* against Colorado potato beetle under heated vs. unheated greenhouse conditions described in Table 2.

Test	Greenhouse condition	Treatment: percent mortality (number of replicate plants) [a,b]			
		Bb C	Bt	Bb	Bt + Bb
Test 1	Heated	10.8 ± 4.4 (6)	66.0 ± 3.3 (5)	13.0 ± 2.5 (5)	83.0 ± 7.7 (5)
Test 2	Heated	15.0 ± 4.8 (6)	79.2 ± 6.1 (6)	35.0 ± 8.0 (6)	95.0 ± 1.8 (6)
Test 3	Unheated	14.4 ± 5.0 (6)	44.0 ± 8.6 (5)	59.3 ± 6.2 (10)	81.3 ± 7.7 (5)
Test 4	Unheated	8.0 ± 3.9 (5)	12.0 ± 3.9 (5)	68.0 ± 7.7 (5)	89.3 ± 5.4 (5)
Test 5	Heated	9.0 ± 1.9 (5)	61.0 ± 6.0 (5)	16.0 ± 4.6 (5)	98.0 ± 2.0 (5)
Mean		11.7 ± 1.9 (28)	53.5 ± 5.3 (26)	41.6 ± 4.8 (31)	89.6 ± 2.5 (26)
Linear regression Percent mortality-Temperature [c]		–	$R^2 = 0.566$ $F_{1,24} = 31.3$ $P < 0.0001$	$R^2 = 0.578$ $F_{1,29} = 39.8$ $P < 0.0001$	$R^2 = 0.065$ $F_{1,24} = 1.7$ $P = 0.207$

[a] For complete description of treatments, see Table 1. Means (± standard errors) are raw means of untransformed, unweighted data.

[b] Tests 1, 3 and 4 with 15 larvae/plant; tests 2 and 5 with 20 larvae/plant.

[c] Mean hourly temperatures recorded in the five tests (Table 2) were nearly perfectly correlated with relative humidity (r = 0.946).

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Table 4. Effects of foliar spray applications of *B. bassiana*- and *B. thuringiensis*-based biopesticides against Colorado potato beetle larvae under greenhouse conditions.

Treatment [a]	Percent mortality (number of replicate plants)	Observed % control (corrected)	Expected % control [b]	Synergism (+) / antagonism (–)	ANOVA main effects and interactions (data empirical-logit transformed and inverse-variance weighted)	
	Raw means ± SE [c]				Random effects	Percent of total variance
Control (Bb C) [c]	11.7 ± 1.9 (28)	–			Test	0%
Bt	53.5 ± 5.3 (26)	47.3			Test x Bt	25.6%
Bb	41.6 ± 4.8 (31)	33.9			Test x Bb	29.0%
Bt + Bb	89.6 ± 2.5 (26)	88.2	65.2	+ 23.0%	Test x Bt x Bb	3.3% [d]
	LS means (95% CI) [c]				[d]Fixed effects	
Control (Bb C)	9.2 (2.6–22.3) (28)	–			Bt	$F_{1,3.9} = 22.8$, P = 0.009
Bt	46.7 (25.4–69.1) (26)	41.3			Bb	$F_{1,3.9} = 12.2$, P = 0.026
Bb	33.2 (15.8–55.9) (31)	26.4			Bt x Bb	$F_{1,101.2} = 7.4$, P = 0.008
Bt + Bb	91.9 (79.2–98.1) (26)	91.1	57.2	+ 33.9%		
	Raw means ± SE					
Control (Bb C)	11.5 ± 3.3 (11)	–				
Bt-L	16.0 2.7 (10)	5.1			Fixed effects [e]	
Bb	62.2 ± 4.8 (10)	57.3			Bt-L	$F_{1,37} = 1.6$, P = 0.21
Bt-L x Bb	67.3 ± 5.2 (10)	63.1	59.5	+ 3.6%	Bb	$F_{1,37} = 102.2$, P < 0.0001
					Bt-L x Bb	$F_{1,37} = 0.57$, P = 0.47

[a] For complete description of treatments, see Table 1.

[b] Control predicted if agents exhibit independent action (estimated from combination of independent probabilities).

[c] Raw means ± standard errors are of untransformed, unweighted data; least-squares (LS) means ± standard errors are from ANOVA; data are back-transformed means and 95% confidence intervals.

[d] The random effect Test x Bt x Bb interaction accounted for only 3.3% of total variance and was removed from final model.

[e] All random effects (RE) and interactions involving RE accounted for a combined total of < 15% of total variance, and were removed from the final Bt-L model.

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Table 5. Effects of foliar spray applications of *B. bassiana*- and *B. thuringiensis*-based biopesticides against Colorado potato beetle larvae under heated vs. unheated greenhouse conditions.

Treatment [a]	Percent mortality [b] (number of replicate plants)	Observed % control (corrected)	Expected % control [c]	Synergism (+) / antagonism (–)	ANOVA main effects and interactions (data empirical-logit transformed and inverse-variance weighted)	
Unheated greenhouse					Random effects [d]	Percent of total variance
Control (Bb C)	11.5 ± 3.3 (11)				Test	3.8%
Bt	28.0 ± 6.9 (10)	18.6			Test x Bt x Bb	3.5%
Bb	62.2 ± 4.8 (15)	57.3			Test x Bb x heated	13.8%
Bt + Bb	85.3 ± 4.6 (10)	83.4	65.2	+ 18.2%		
					Fixed effects	
Heated greenhouse					Heated	$F_{1,2.97} = 0.03$, P = 0.87
Control (Bb C)	11.8 ± 2.3 (17)	–			Bt	$F_{1,101.8} = 149.3$, P < 0.0001
Bt	69.4 ± 3.6 (16)	65.3			Bb	$F_{1,101} = 136.8$, P < 0.0001
Bb	22.2 ± 4.1 (16)	11.8			Heated x Bt	$F_{1,101.8} = 32.6$, P < 0.0001
Bt + Bb	92.2 ± 2.9 (16)	91.2	69.4	+ 21.8%	Heated x Bb	$F_{1,101} = 26.3$, P < 0.0001
					Bt x Bb	$F_{1,101.4} = 6.6$, P = 0.012
					Heated x Bt x Bb	$F_{1,101.4} = 0.51$, P = 0.48

[a] For complete description of treatments, see Table 1.

[b] Raw means ± standard error (means of untransformed, unweighted data).

[c] Control predicted if agents exhibit independent action.

[d] Only one interaction involving a random effect (Test x heated x Bb) accounted for > 10% of total variance. Test was ultimately retained in the final ANOVA only as a main-effect blocking factor.

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Table 6. Effects of foliar spray applications of *B. bassiana*- and *B. thuringiensis*-based biopesticides and spray carriers against Colorado potato beetle larvae in a July 2005 field trial.

Treatment [a]	Larvae per 10 stems [b] (number of replicate field plots)	Observed % control	Expected % control [c]	Synergism (+) / antagonism (–)	ANOVA main effects and interactions (data log-transformed)	
Controls	Raw means ± SE [d]				Full model	
UT	94.9 ± 22.8 (4)				Random effect [e]	Percent of total variance
Bt C	109.0 ± 7.7 (4)				Field block	53.1%
Bb C	107.7 ± 22.8 (4)				Fixed effects	
Bt C + Bb C	102.8 ± 3.3 (4)				Bt	$F_{1,19.1} = 91.8$, $P < 0.0001$
Treatments					Bb	$F_{1,19.1} = 8.2$, $P = 0.001$
Bt	43.2 ± 7.1 (4)				Bt x Bb	$F_{1,19.1} = 10.9$, $P = 0.004$
Bt + Bb C	42.8 ± 7.9 (4)				Bt C	$F_{1,19.1} = 1.6$, $P = 0.23$
Bb	114.9 ± 7.6 (4)				Bb C	$F_{1,19.1} = 2.1$, $P = 0.16$
Bb + Bt C	122.3 ± 11.8 (4)				Bt x Bb C	$F_{1,19.1} = 0.91$, $P = 0.35$
Bt + Bb	22.3 ± 5.9 (4)				Bb x Bt C	$F_{1,19.1} = 0.03$, $P = 0.86$
					Bt C x Bb C	$F_{1,19.1} = 1.3$, $P = 0.26$
Combined treatments [f]						
Controls	103.6 ± 7.6 (16)					
Bt	43.0 ± 4.9 (8)	58.5			Comparing controls	Percent of total variance
Bb	118.6 ± 6.7 (8)	0			Random effect	67.0%
Bt + Bb	22.3 ± 5.9 (4)	78.5	58.5	+ 20%	Field block	
					Fixed effect	
	LS means ± SE [d]				Treatment	$F_{3,6.5} = 0.37$, $P = 0.56$
Controls	2.003 ± 0.111 [100.6]					
Bt	1.585 ± 0.155 [38.4]	61.8				
Bb	2.032 ± 0.155 [107.8]	0				
Bt + Bb	1.173 ± 0.244 [14.9]	85.2	61.8	+23.4%		

[a] For complete description of treatments, see Table 1.

[b] Mean larvae per 10 stems per replicate field plot per sample date (samples collected 7, 10, 13, and 15 days after initial application).

[c] Control predicted if agents exhibit independent action (estimated from combination of independent probabilities).

[d] Raw means ± standard errors are of untransformed, unweighted data; least-squares (LS) means are from ANOVA; numbers in brackets are the back-transformed means.

[e] Interactions involving random effect Field block were not tested (see Sokal and Rohlf, 1995).

[f] Combined control (UT, Bt C, Bb C, and Bt C + Bb C), combined Bt (Bt and Bt + Bb C), and combined Bb (Bb and Bb +Bt C) treatments.

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Table 7. Effects of foliar spray applications of *B. bassiana*- and *B. thuringiensis*-based biopesticides and spray carriers against Colorado potato beetle larvae in a July 2006 field trial.

Treatment [a]	Larvae per 10 stems [b] (number of replicate field plots)	Observed % control	Expected % control [c]	Synergism (+) / antagonism (–)	ANOVA main effects and interactions (log-transformed data)	
					Full model	
Controls	Raw means ± SE [d]				Random effect [e]	Percent of total variance
UT	68.2 ± 3.3 (4)				Field block	40.8%
Bt C	58.4 ± 2.8 (4)				Fixed effects	
Bb C	60.3 ± 5.9 (4)				Bt	$F_{1,19.8} = 158.0$, $P < 0.0001$
Bt C + Bb C	57.0 ± 3.4 (4)				Bb	$F_{1,19.8} = 87.7$, $P < 0.0001$
Treatments					Bt x Bb	$F_{1,19.8} = 30.5$, $P < 0.0001$
Bt	30.1 ± 3.4 (4)				Bt C	$F_{1,19.8} = 0.08$, $P = 0.78$
Bt + Bb C	29.6 ± 3.4 (4)				Bb C	$F_{1,19.8} = 1.1$, $P = 0.30$
Bb	37.8 ± 3.9 (4)				Bt x Bb C	$F_{1,19.8} = 0.05$, $P = 0.83$
Bb + Bt C	45.9 ± 4.7 (4)				Bb x Bt C	$F_{1,19.8} = 0.73$, $P = 0.40$
Bt + Bb	8.4 ± 1.8 (4)				Bt C x Bb C	$F_{1,19.8} = 0.02$, $P = 0.90$
Combined treatments [f]						
Controls	61.0 ± 2.1 (16)	–			Comparing controls	
Bt	29.8 ± 2.2 (8)	51.2			Random effect	Percent of total variance
Bb	41.8 ± 3.2 (8)	31.5			Field block	0%
Bt + Bb	8.4 ± 1.8 (4)	86.2	64.2	+ 22%	Fixed effect	
					Treatment	$F_{3,11.4} = 0.71$, $P = 0.56$
	LS means ± SE [d]					
Controls	1.784 ± 0.059 [60.8]	–				
Bt	1.498 ± 0.089 [31.5]	48.2				
Bb	1.628 ± 0.089 [42.5]	30.1				
Bt + Bb	0.895 ± 0.145 [7.9]	87.1	67.4	+19.7%		

[a] For complete description of treatments, see Table 1.

[b] Mean larvae per 10 stems per replicate field plot per sample date (samples collected 8, 12, 14, and 15 days after initial application).

[c] Control predicted if agents exhibit independent action (estimated from combination of independent probabilities).

[d] Raw means ± standard errors are of untransformed, unweighted data; least-squares (LS) means ± standard errors are from ANOVA; numbers in brackets are the back-transformed means.

[e] Interactions involving random effect Field block were not tested (see Sokal and Rohlf, 1995).

[f] Combined control (UT, Bt C, Bb C, and Bt C + Bb C), combined Bt (Bt and Bt + Bb C), and combined Bb (Bb and Bb + Bt C) treatments.

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Table 8. Effects of foliar spray applications of *B. bassiana*- and *B. thuringiensis*-based biopesticides and spray carriers on next-generation adult Colorado potato beetle populations in a July 2006 field trial.

Treatment a	Adults per 18 plants b (number of replicate field plots)	Observed % control	Expected % control [c]	Synergism (+) / antagonism (–)	ANOVA main effects and interactions (log-transformed data)	
					Full model	
Controls	Raw means ± SE [d]				Random effect [e]	Percent of total variance
UT	217.5 ± 72.5 (4)				Field block	50.9%
Bt C	189.8 ± 67.0 (4)				Fixed effects	
Bb C	162.3 ± 71.0 (4)				Bt	$F_{1,18.9} = 26.0$, $P < 0.0001$
Bt C + Bb C	240.5 ± 78.8 (4)				Bb	$F_{1,18.9} = 26.4$, $P < 0.0001$
Treatments					Bt x Bb	$F_{1,19.8} = 30.5$, $P < 0.0001$
Bt	9.3 ± 2.8 (4)				Bt carrier	$F_{1,19.8} = 0.60$, $P = 0.45$
Bt + Bb C	10.8 ± 4.5 (4)				Bb carrier	$F_{1,18.9} = 0.01$, $P = 0.91$
Bb	10.7 ± 3.8 (4)				Bt x Bb carrier	$F_{1,18.9} = 0.05$, $P = 0.83$
Bb + Bt C	7.5 ± 3.4 (4)				Bb x Bt carrier	$F_{1,18.9} = 4.0$, $P = 0.0605$
Bt + Bb	4.0 ± 1.2 (4)				Bt carrier x Bb carrier	$F_{1,19.8} = 0.15$, $P = 0.70$
Combined treatments [f]						
Controls	202.5 ± 33.3 (16)	–			Comparing controls	
Bt	10.0 ± 2.4 (8)	95.1			Random effect	Percent of total variance
Bb	9.1 ± 2.4 (8)	95.5			Field block	82.7%
Bt + Bb	4.0 ± 1.2 (4)	98.0	99.8	- 1.8%	Fixed effects	
					Treatment	$F_{3,4.8} = 0.49$, $P = 0.71$
	LS means ± SE [d]					
Controls	2.189 ± 0.248 [154.7]	–				
Bt	0.836 ± 0.352 [6.9]	95.5				
Bb	0.831 ± 0.352 [6.8]	95.6				
Bt + Bb	0.618 ± 0.558 [4.1]	97.3	99.8	- 2.5%		

[a] For complete description of treatments, see Table 1.

[b] Mean adults per 18 stems per replicate field plot (adults counted 25 days after initial application).

[c] Control predicted if agents exhibit independent action (estimated from combination of independent probabilities).

[d] Raw means ± standard errors are of untransformed, unweighted data; least-squares (LS) means ± standard errors are from ANOVA; numbers in brackets are the back-transformed means.

[e] Interactions involving random effect Field block were not tested (see Sokal and Rohlf, 1995).

[f] Combined control (UT, Bt C, Bb C, and Bt C + Bb C), combined Bt (Bt and Bt + Bb C), and combined Bb (Bb and Bb + Bt C) treatments.

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