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Synergistic interaction between *Beauveria bassiana*- and *Bacillus thuringiensis tenebrionis*-based biopesticides applied against field populations of Colorado potato beetle larvae ☆

S.P. Wraight *, M.E. Ramos

USDA-ARS, U.S. Plant, Soil and Nutrition Laboratory, Tower Road, Ithaca, NY 14853, USA

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Abstract

Commercial biopesticides based on the fungal pathogen *Beauveria bassiana* strain GHA and the bacterial pathogen *Bacillus thuringiensis tenebrionis* were applied alone and in combination (tank mixed) against larval populations of the Colorado potato beetle, *Leptinotarsa decemlineata*, in small plots of potatoes over three field seasons. Interactions between the two products were evaluated in terms of pest-control efficacy. *B. bassiana* (formulated as Mycotrol) was applied at low and medium label rates of 1.25 and 2.5×10^{13} conidia/ha, and *B. thuringienis* (formulated as Novodor) was applied at low and high label rates of 40.3 and 120.8×10^{6} Leptinotarsa units/ha. Two weekly applications of the bacterial pesticide alone provided 50–85% control of beetle larvae within 14 days after the initial application, while applications of the mycopesticide alone produced no greater than 25% control. Maximum control, in nearly all tests, was produced by the combination of the two products. The combined treatments produced a statistically significant 6–35% greater reduction in larval populations than would have been predicted had the two biopesticides acted independently. This low-level synergistic interaction was observed during all field seasons and resulted from combinations at all rates, including, in one of two tests, the low rates of each product. These results indicate that *B. thuringiensis* and *B. bassiana* have strong potential for integrated biologically based management of Colorado potato beetle.

Keywords: *Bacillus thuringiensis tenebrionis*; *Beauveria bassiana*; *Leptinotarsa decemlineata*; Colorado potato beetle; Biopesticides; Interaction; Synergism

1. Introduction

The Colorado potato beetle, *Leptinotarsa decemlineata* Say (Coleoptera: Chrysomelidae), is one of agriculture's most intractable pests, having exhibited a remarkable capacity to resist nearly every chemical insecticide applied against it, in many cases within only a few growing seasons (Forgash, 1981; Storch, 1995). The problems of insecticide resistance, combined with continuing environmental concerns associated with chemical pesticide use, have provided considerable stimulus over the past 50 years for development of alternative control methods, including mechanical, physical, and biological control. This work continues to the present, even though adoption of nonchemical control agents and strategies by farmers has been limited by repeated, timely introductions of novel, highly efficacious, and economical chemical insecticides.

The well-known fungal pathogen, *Beauveria bassiana* (Balsamo) Vuillemin (Hyphomycetes), is a ubiquitous and important natural enemy of *L. decemlineata* (Humber, 1996; Roberts et al., 1981). Commercial-scale use of this pathogen for potato beetle control was pioneered in the former Soviet Union and Eastern Europe. The biopesticide Boverin was developed in the early 1960s and produced in large quantities over the following two decades (Deacon, 1983; Roberts et al., 1981). This work ultimately stimulated

☆ Mention of trade names or commercial products in this publication is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the US Department of Agriculture.

* Corresponding author. Fax: +1 607 255 1132.
E-mail address: spw4@cornell.edu (S.P. Wraight).

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similar development efforts in other countries, including France in the 1970s and the USA during the 1980s. This work has considerable scientific and historic significance with respect to the fields of applied insect pathology and microbial control; however, the success of these *B. bassiana*-based control programs was inconsistent. Foliar applications provided slow and inadequate control of high-density larval populations and of late-instar larvae and adults (Lipa, 1985). Based on simulation modeling, recommendations were ultimately developed that called for frequent applications (at 3- to 4-day intervals) against early instar larvae (Galaini, 1984); however, results under this protocol also have been highly variable (cf. Hajek et al., 1987; Poprawski et al., 1997; Wraight and Ramos, 2002). Researchers have consequently concluded that *B. bassiana* alone cannot be relied upon to provide sufficiently high levels of control within the brief period of time necessary to protect potatoes from economic damage.

Soviet scientists recognized this problem early in the development process and in the mid 1960s began to study efficacy of *B. bassiana* combined with reduced rates of chemical insecticides. Pest control researchers reported successful control from combinations of Boverin with 1/4 rates of DDT and other insecticides (see Lipa, 1985). The explanation of this success was described in terms of the intoxicated (weakened) larvae becoming more susceptible to fungal infection. This work marked the beginning of large-scale efforts to synergize the actions of fungal pathogens with low doses of chemical insecticides.

Few of the early studies that reported synergistic interactions involving pathogens included data or data analyses sufficiently rigorous to support the claims (Benz, 1971), yet there seems little doubt that synergism played at least some role in the potato beetle trials reported by Soviet and Polish researchers (see reviews by Ferron, 1985; Telenga, 1962). Jaworska (1987) suggested that the strong synergism reported from the initial field applications of *B. bassiana* with low doses of insecticides in Eastern Europe and the Soviet Union occurred because the beetle populations had not yet developed insecticide resistance. A more recent report indicates that *B. bassiana* activity against Colorado potato beetle is synergized by low doses of imidacloprid (Furlong and Groden, 2001).

Toxins of *B. thuringiensis* Berliner have been applied against Colorado potato beetle since the mid 1970s. A preparation of *B. thuringiensis* containing thuringiensis (β-exotoxin) as the active ingredient was produced in the Soviet Union under the name Bitoxibacillin (Lipa, 1985). Experimental thuringiensin-based preparations also have been tested in the USA (Anderson et al., 1989; Jaques and Laing, 1989). However, the broad-spectrum toxicity of thuringiensin, combined with its teratogenic and mutagenic properties, has limited commercial development. A subspecies of *B. thuringiensis* that produces δ-endotoxins (cryIIIA and cryIIIBb) with high activity against many beetles was discovered by Krieg et al. (1983). This pathogen, *B. t. tenebrionis* was rapidly developed and incorporated into commercial biopesticide formulations for control of *L. decemlineata*. Because the beetle larvicidal activity of *B. t. tenebrionis* is based on a more host-specific toxin (δ-endotoxin) and because important effects of intoxication include reduced activity and slowed development of the host, this agent has been identified as having potential to synergize a broad range of potato beetle predators and parasites (Cloutier et al., 1995).

This action also suggests that *B. thuringiensis* might be applied in concert with other insect pathogens, including entomopathogenic fungi. There is, however, little known with regards to interactions between *B. thuringiensis* and entomopathogenic fungi (Navon, 2000). Sandner and Cichy (1967) applied a mixture of *B. thuringiensis kurstaki* and *B. bassiana* against larvae of the Mediterranean flour moth. Results of a single laboratory experiment indicated that the two agents acted independently (mean mortalities from *B. bassiana*, *B. thuringiensis*, and the mixture were 57, 44, and 71%, respectively). Anderson et al. (1989) included the toxin thuringiensin in their studies of interactions between *B. bassiana* and chemical insecticides applied against *L. decemlineata*. It was concluded that these agents did not act synergistically; however, the data were inadequate for rigorous testing of interactions. Lewis and Bing (1991) applied *B. thuringiensis kurstaki* in combination with *B. bassiana* in a granular formulation against European cornborer. The authors concluded that the results, expressed in terms of crop damage (stalk tunneling), indicated independent action. In this test, however, the dose of the fungus was low, and its addition to the *B. thuringiensis* treatments actually had no significant effects. Because the fungus applied alone caused a significant reduction in damage, such a response to the combined agents could only be explained by antagonism (a lower response than would be expected from independent action). Costa et al. (2001) also observed no synergistic effect when fourth-instar Colorado potato beetle larvae that survived *B. thuringiensis* intoxication were treated with *B. bassiana*.

In this study, we examined interactions between *B. bassiana*- and *B. thuringiensis tenebrionis*-based biopesticides applied as mixtures against field populations of *L. decemlineata* during three field seasons.

2. Materials and methods

The studies were conducted in small, replicated field plots on Cornell University's H. C. Thompson Vegetable Crops Research Farm in Freeville, New York, and were initiated through an informal collaboration between the USDA-ARS and Mycotech Corporation of Butte MT.

2.1. Biopesticide preparations

All fungal formulations were produced at the production facility of Mycotech Corp. in Butte, Montana (now Emerald BioAgriculture Corp.) utilizing proprietary methods and ingredients (see Bradley et al., 1992). Strain GHA

of *B. bassiana* (Bradley et al., 2001) was formulated as an oil-based emulsifiable suspension (Mycotrol ES) containing 2.1×10^{10} conidia/ml and as a clay-based wettable powder (Mycotrol 22WP) containing 4.4×10^{10} conidia/g. All preparations were stored at 4 °C and retained high initial viability (>93%) for the duration of the study.

Conidial concentrations in the unformulated conidial powders used to prepare the WP and ES formulations were determined by preparing aqueous suspensions (1–3 mg powder/ml) and counting spores at 400× magnification in standard (improved Neubauer) hemacytometer chambers. Viability of conidia was determined by direct observation of conidia (400× magnification) plated on agar containing yeast extract (0.5%) and incubated 16–18 h at 25 °C. All conidia with visible germ tubes of any length were scored as viable. The amounts of mycopesticide product necessary to achieve the desired rates (viable conidia/ha) in a specific spray volume were determined from these spore concentration and viability data.

Bacillus thuringiensis tenebrionis, formulated as a flowable concentrate (Novodor FC containing 17,224 Leptinotarsa units/ml), was produced by Abbott Laboratories, Inc. of North Chicago, Illinois and stored at room temperature until use.

2.2. Chemical pesticides

Occurrence in Central New York of hypervirulent strains of the late-blight pathogen, *Phytophthora infestans* (Montagne) de Bary, made prophylactic applications of fungicides mandatory (especially to protect experimental crops on adjacent fields). Fungicides were applied when weather conditions favored late-blight development. Materials and rates used included: Penncozeb DF (Elf Atochem, Philadelphia, PA) at 2.24 kg (1.68 kg maneb)/ha; Dithane M-45 (Rohm and Haas, Philadelphia, PA) at 2.24 kg (1.79 kg mancozeb)/ha; Manzate 200 (DuPont Agricultural Products, Wilmington, DE) at 2.24 kg (1.68 kg mancozeb)/ha; and Ridomil Bravo 81W (Novartis Crop. Protection, Greensboro, NC) at 2.24 kg (0.2 kg metalaxyl + 1.61 kg chlorothalonil)/ha. Monitor 4 (Bayer, Kansas City, MO) was applied at 1.17 L (0.56 kg methamidophos)/ha for control of potato leafhopper, *Empoasca fabae* (Harris).

Fungicides and insecticides were applied using a conventional hydraulic sprayer configured for above-canopy sprays, with TeeJet (Spraying Systems, Wheaton, IL) TJ60-8006EVS spray nozzles spaced 50.8 cm apart. Spray volume was 170 L/ha applied at a pressure of 3.1 bar.

2.3. Field tests

2.3.1. 1998

Potatoes ('Allegheny') were planted 15 May in plots measuring 6 rows × 9.1 m with rows spaced 86 cm. Applications were made using a single-row, backpack, CO_2 hydraulic sprayer (R&D Sprayers, Opelousas, LA) fitted with two TeeJet (Spraying Systems, Wheaton, IL) TXVS-8 hollow-cone spray nozzles mounted on swivels affixed to lateral drop tubes. The nozzles were carried 15–20 cm above the ground and were directed at a 45° angle to spray upward into the potato canopy, maximizing coverage of ventral leaf surfaces (Wraight and Ramos, 2002). The sprayer delivered 280 L/ha at 3.45 bar. Each treatment was applied to five replicate plots. Two applications were made, 8 days apart on 1 and 9 July. A summary of the treatments is presented in Table 1. Manzate 200 was applied for late-blight control on 2, 15, and 21 July, and Monitor 4 was applied for leafhopper control on 15 July.

2.3.2. 1999

Potatoes ('Salem') were planted 17 May in plots measuring 6 rows × 7.6 m with rows spaced 86 cm. Treatments (Table 1) were applied as in 1998. Each treatment was applied to six replicate plots. Two applications were made 6 days apart on 29 June and 5 July. Dithane M-45 was applied on 12 July for late-blight control.

2.3.3. 2000

Potatoes ('Keuka Gold') were planted 17 May in plots of the same size as used in 1999. We decided in 2000 to switch from the backpack sprayer used in 1998 and 1999 to a much larger and more conventional sprayer to demonstrate relevance of our findings to commercial-scale potato production systems. Treatments (Table 1) were applied using a tractor-mounted hydraulic sprayer configured in a manner designed to maximize coverage of the undersides of the crop foliage (Wraight and Carruthers, 1999). Albuz lilac ceramic hollow cone nozzles (Saint Gobain Ceramiques,

Table 1
Beauveria bassiana and *B. thuringiensis tenebrionis* treatments applied to research plots of potatoes infested with Colorado potato beetles during three consecutive field seasons

Treatment[a,b]	Code	Year applied
Untreated control	Untreated	1998, 1999, 2000
B. bassiana ES formulation carrier control[c]	Bb-ES carrier control	1998, 1999, 2000
B. bassiana 22WP formulation carrier control[c]	Bb-WP carrier control	2000
B. bassiana ES; low label rate	Bb-ES-L	1998, 2000
B. bassiana ES; medium label rate	Bb-ES-M	1998, 1999, 2000
B. bassiana 22WP; medium label rate	Bb-WP-M	2000
B. thuringiensis FC formulation; low label rate	Bt-L	1998, 1999, 2000
B. thuringiensis FC formulation; high label rate	Bt-H	1998

[a] *B. bassiana* formulated as Mycotrol ES or 22WP (low and medium label rates = 1.25 and 2.5×10^{13} conidia/ha, respectively); *B. thuringiensis tenebrionis* formulated as Novodor FC (low and high label rates = 40.3 and 120.8×10^6 Leptinotarsa units/ha, respectively).

[b] Fungus + bacterium treatments (not shown) were prepared by simply combining (tank mixing) the indicated treatments.

[c] Carrier controls: ES formulation blank was applied at the medium rate (1.17 L/ha) in 1999 and 2000 and at 2× the medium rate (2.34 L/ha) in 1998 (see Wraight and Ramos, 2002); WP formulation blank was applied at the medium rate (0.56 kg/ha) in 2000.

Vincennes, France) were mounted on swivels on short drop tubes spaced 21.5 cm apart. The nozzles were carried at canopy height and directed forward and downward at a 45° angle to the ground. The sprayer was operated at a pressure of 27.6 bar at a ground speed of 4.8 km/h and delivered a volume of 467.5 L/ha. Each treatment was applied to five replicate plots. Two applications were made, 7 days apart on 29 June and 6 July. Fungicide applications included Ridomil Bravo 81W on 28 June and 5 and 19 July, and Penncozeb DF on 12 July.

2.3.4. Sampling protocols

Leaf samples were collected within 1 day prior to the initial application and for 2–3 weeks at irregular intervals of 1–7 days thereafter. On each sample date, 10 stems (20–25 cm in length) were collected from random locations within each replicate plot. All collected leaves were held in plastic bags at 4 °C prior to processing. All live larvae on the 10 stems collected from each plot were counted and categorized as early (first and second) or late (third and fourth) instars.

2.3.5. Defoliation and yield determinations

Defoliation was assessed during the 1998 and 1999 seasons approximately 2 weeks after the initial application. Individual potato plants were examined at 15 randomized locations in each plot in 1998 and at 10 locations per plot in 1999, and the level of defoliation at each location was visually estimated to the nearest 10%. Defoliation on a per plot basis was estimated as the average of the 10 or 15 readings. In late September 1999, all potatoes were harvested from each plot and weighed. In mid October 2000, potatoes were collected from the central 2-m section of each plot (total of 12 row meters) and weighed. Yield was expressed as total kilograms of potatoes per meter of row. Low beetle populations inflicted only minor damage in 1998 and 2000 (<10% defoliation in the control plots). As a consequence, yields were not recorded in 1998 and defoliation was not measured in 2000.

2.3.6. Environmental monitoring

Rainfall was monitored using portable electronic data loggers (Omnidata International, Logan, Utah) maintained in the test field. Air temperature, relative humidity, and solar radiation data were obtained from the Northeast Regional Climate Center at Cornell University (weather station located approximately 13 km from the field site). The Freeville field site and the weather station are located at comparable elevations (319 and 293 m, respectively).

2.3.7. Experimental design and statistical analyses

All plots in each test were arranged in a randomized, complete block design (one replicate plot per block). Two-way ANOVAs were conducted using the JMP statistical software (SAS Institute, 1995). Analyses included nominal variables representing application or no application of *B. thuringiensis* and *B. bassiana* treatments. Colorado potato beetle larval population responses to the multiple spray treatments were, in most cases, expressed gradually over extended periods of time, and a simple approach was adopted for evaluating differences in treatment effects. Analyses were conducted on means derived from the grand total larvae recorded on all stems collected over a specified period of time from each plot. Treatment means were thus based on 5–6 values (one from each block) regardless of the number of sample dates included. The logarithmic transformation was applied to all beetle numbers subjected to ANOVA. Data sets including zero counts were transformed to $\log(n+1)$. Synergism was identified on the basis of a statistically significant interaction between the main effects (fungus and bacterium) (Sokal and Rohlf, 1995).

The 1998 and 1999 tests were conducted at the same time and in the same fields as tests described in our previous paper (Wraight and Ramos, 2002). The treatments described here were incorporated (strictly randomized) into the tests described in the previous paper and utilized the same sets of control plots. Also, drought conditions severely limited field space in 1999, and it was not possible to establish an independent set of plots for the *B. bassiana*-alone treatment (the ES-M-BC-7d treatment of Wraight and Ramos (2002)). Moreover, in the 1998 and 2000 field tests, we were not able to establish and monitor an entirely independent set of treatments for each of the multiple synergism investigations (using different doses of the pathogens). The same controls and some of the same *B. thuringiensis* and *B. bassiana* treatments are therefore compared in the different ANOVAs (see Tables 2 and 4). The sharing of treatments between experiments is an efficient approach for exploring a large number of treatments in costly field tests; however, the sacrifice of independence in some multiple comparisons increases the experimentwise error rate (Sokal and Rohlf, 1995). This problem was compensated for in several ways: (1) by including more than one set of control treatments in each test (maximizing confidence in the estimate of the natural (untreated) population), (2) by repeating the tests over three field seasons, and (3) by conducting overall analyses that combined all treatments and controls from a single season where possible. It should also be noted that all treatment comparisons made here and in Wraight and Ramos (2002) were planned (a priori) and limited to considerably fewer than all possible pairwise comparisons. Finally, the 2000 test reported herein was conducted independently of other experiments.

In all following presentations of results, reports of percent control or percent reduction of larval populations indicates the percent decrease in the population relative to the control treatments. In the following discussions, all references to days indicate days after the initial fungus application.

3. Results

3.1. Larval populations

No differences in larval populations were observed between the untreated and carrier control treatments

during any of the three field seasons. In all cases, orthogonal comparisons of the numbers of larvae collected from the different controls from approximately day 7 through the end of each test yielded P values >0.50. In light of this result, all control treatments from each test were pooled to provide a single estimate of the untreated (natural) population density. This estimate is labeled "controls" in the figures and tables (as per Wraight and Ramos, 2002).

3.2. 1998 Field season

Due to persistent heavy rains, application was delayed for 6 days beyond egg hatch, and by the day of the first application (1 July), the larval population comprised 84.1% late instars (primarily third instars). Under these conditions, the applications of both the low and medium rates of the *B. bassiana* ES formulation alone produced no significant population reductions (Fig. 1 and Table 2). Applications of the *B. thuringiensis* product alone, on the other hand, reduced larval numbers by 31–46% within 7 days (pooled low and medium rates: $F_{[1,14]}=4.6$; $P=0.050$) and 55–56% within 11 days ($F_{[1,14]}=6.1$; $P=0.027$).

On day 16, the larval population treated with the low rates of the *B. thuringiensis* and *B. bassiana* products combined was numerically lower than the population treated with the most active agent (the low rate of *B. thuringiensis* alone) (Fig. 1A), but the difference was not significant ($F_{[1,4]}=3.2$; $P=0.147$). The population exposed to the combined treatment increased over the next 7 day (an apparent sampling error), and the ANOVA showed no significant synergistic interaction between the two biopesticides ($P=0.563$; Table 2).

The combined low rate of *B. thuringiensis* and medium rate of *B. bassiana* caused a significantly greater reduction in larval numbers than the low rate of *B. thuringiensis* alone (days 11–23 $F_{[1,4]}=17.0$; $P=0.015$), and in this case, the combined treatments resulted in a 35% greater reduction than would be expected from independent action (the interaction P was 0.006) (Table 2). A similar result was obtained when the high rate of *B. thuringiensis* was applied. Analysis showed that populations treated with the combined biopesticides were lower than those treated with the bacterial product alone (days 7–23 $F_{[1,4]}=24.6$; $P=0.008$), and the interaction P was 0.004 (Table 2).

The overall ANOVA (including all treatments) verified a synergistic interaction between the fungal and bacterial preparations ($P=0.027$; Table 2).

3.3. 1999 Field season

The 1999 field test was initiated under severe drought conditions, which limited the extent of testing. The plants emerged unevenly and were stunted. At the time of initial application, 91% of the larvae were first or second instars.

As was observed in 1999, treatment with the fungal product alone provided no significant control of beetle larvae (days 7–14 $F_{[1,5]}=2.5$; $P=0.141$) (Fig. 2). At the same time, applications of *B. thuringiensis* alone reduced larval populations by >85% within 7 days. Samples collected between days 8 and 14 showed greater efficacy of the combined fungus-bacterium treatment compared to the bacterium treatment alone ($F_{[1,5]}=9.6$; $P=0.027$). The difference between the population densities (2.5 versus 1.0 larvae/10 stems/sample date) was significant even though the

Table 2
Effects of foliar spray applications of *B. bassiana*- and *B. thuringiensis tenebrionis*-based biopesticides against Colorado potato beetle larvae in a July 1998 field trial

Treatment[a]	Larvae[b]	Observed % control	Expected[c] % control	Synergism (+) or antagonism (−)	ANOVA main effects and Interactions	
Controls	7.1 ± 0.83	—			Bt	$F_{[1,17]}=11.7$; $P=0.003$
Bt-L	4.0 ± 0.58	43.7			Bb	$F_{[1,17]}=0.29$; $P=0.599$
Bb-ES-L	9.3 ± 2.43	0.0			Bt × Bb	$F_{[1,17]}=0.35$; $P=0.563$
Bt-L + Bb-ES-L	4.2 ± 0.83	40.8	43.7	−2.9 %		
Controls	7.1 ± 0.83	—			Bt	$F_{[1,17]}=36.8$; $P<0.0001$
Bt-L	4.0 ± 0.58	43.7			Bb	$F_{[1,17]}=7.9$; $P=0.012$
Bb-ES-M	7.6 ± 1.32	0.0			Bt × Bb	$F_{[1,17]}=10.0$; $P=0.006$
Bt-L + Bb-ES-M	1.5 ± 0.28	78.9	43.7	+35.2 %		
Controls	7.1 ± 0.83	—			Bt	$F_{[1,17]}=65.8$; $P<0.0001$
Bt-H	3.4 ± 1.06	52.1			Bb	$F_{[1,17]}=8.9$; $P=0.008$
Bb-ES-M	7.6 ± 1.32	0.0			Bt × Bb	$F_{[1,17]}=11.0$; $P=0.004$
Bt-H + Bb-ES-M	1.0 ± 0.26	85.9	52.1	+33.8 %		
Controls	7.1 ± 0.83	—			Bt	$F_{[1,37]}=38.7$; $P<0.0001$
All Bt	3.7 ± 0.58	47.9			Bb	$F_{[1,37]}=2.4$; $P=0.130$
All Bb	8.4 ± 1.34	0.0			Bt × Bb	$F_{[1,37]}=5.3$; $P=0.027$
All Bt + Bb	2.2 ± 0.47	69.0	47.9	+21.1 %		

[a] For complete description of treatments, see Table 1.
[b] Mean larvae per 10 stems per sample date from four consecutive samples collected 11, 12, 16, and 23 days after initial application (± standard error; controls, $n=10$; individual treatments, $n=5$; all Bt and all Bb, $n=10$; all Bt + Bb, $n=15$).
[c] Control predicted if the agents exhibit independent action.

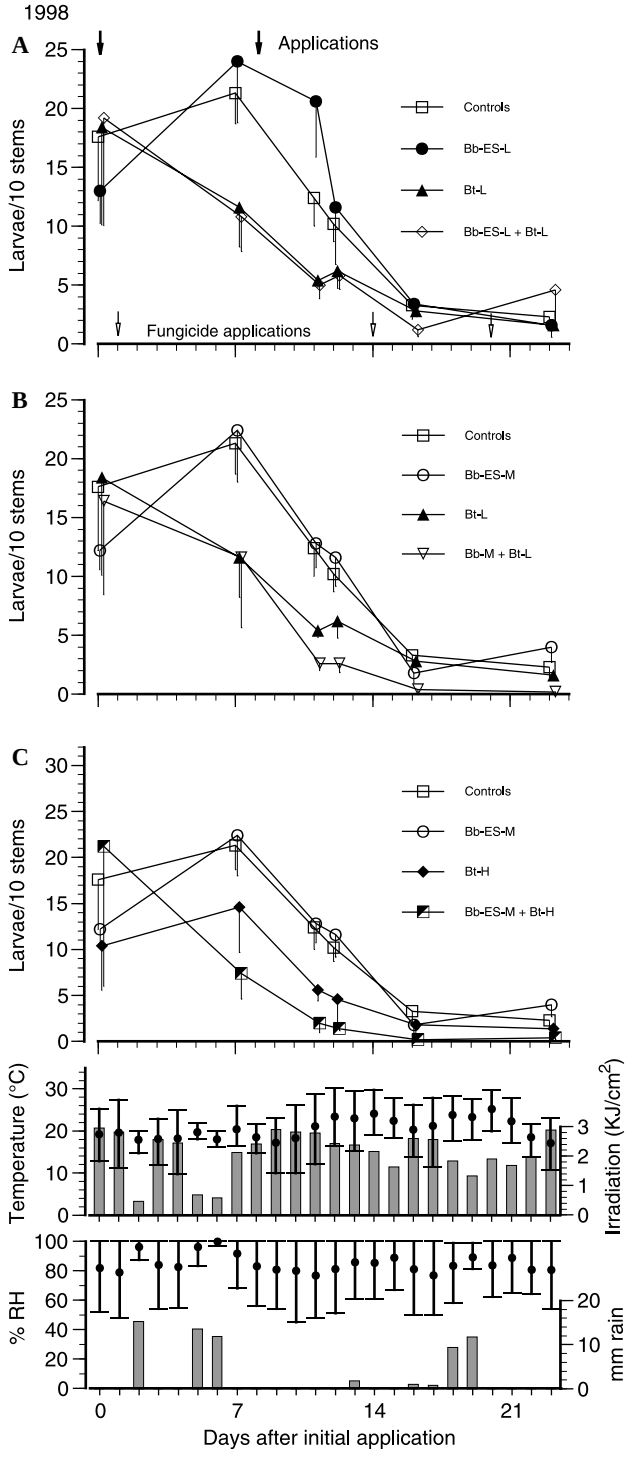

Fig. 1. Trends in larval Colorado potato beetle populations during a program of spray applications of *B. bassiana*- and *B. thuringiensis tenebrionis*-based biopesticides during a 1998 field trial with moisture and temperature data. Explanation of treatment codes in figure legend is presented in Table 1. Vertical lines represent standard errors of means; for clear presentation of standard errors, some means are offset on the *x* axis.

difference in percent control provided by the two treatments was small (88 versus 95%). ANOVA also revealed a statistically significant interaction ($P = 0.006$; Table 3). Mortality was 6% greater than predicted by independent action.

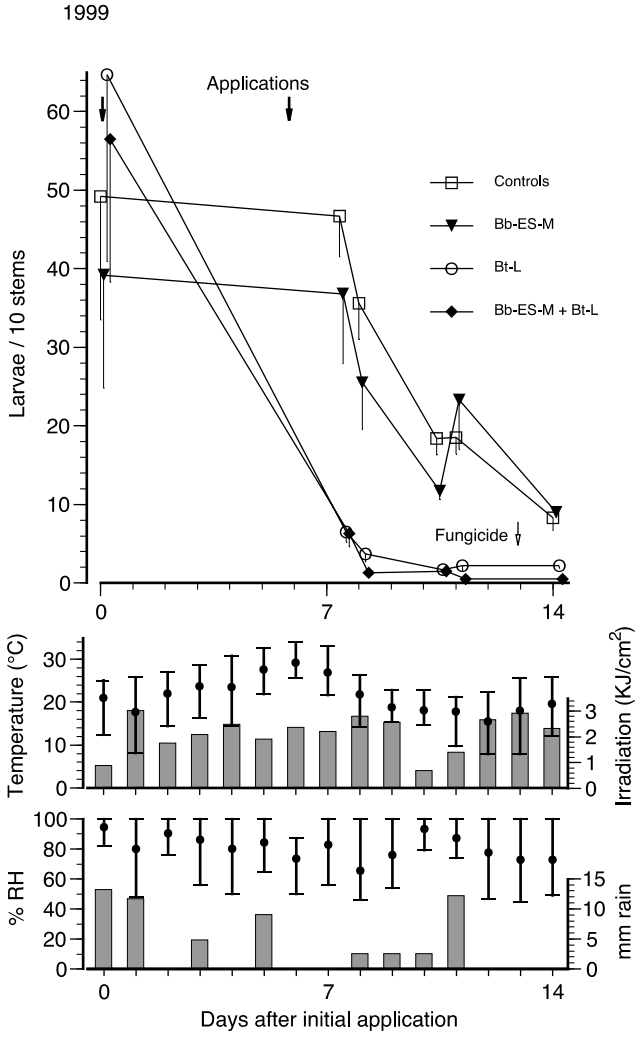

Fig. 2. Trends in larval Colorado potato beetle populations during a program of spray applications of *B. bassiana*- and *B. thuringiensis tenebrionis*-based biopesticides during a 1999 field trial with moisture and temperature data. Explanation of treatment codes in figure legend is presented in Table 1. Vertical lines represent standard errors of means; for clear presentation of standard errors, some means are offset on the *x* axis.

3.4. 2000 Field season

At the time of the initial application, 96% of the beetle larvae were first or second instars. As was observed in 1998, the low rate of the *B. bassiana* ES formulation applied alone had virtually no impact on the larval populations (Fig. 3A). The low rate of the *B. thuringiensis* formulation, on the other hand, reduced the population by 50% within 7 days and nearly 80% within 9 days. Larval numbers in the combined fungus-bacterium treatment were lower than those in the bacterium-alone treatment beginning on day 5 and continuing over nearly the entire course of the experiment (days 5–21 $F_{[1,4]} = 47.0$; $P = 0.002$). The ANOVA applied to samples collected 12–19 days after initial treatments yielded an interaction P value of 0.032; mortality was 16% greater than expected from independent action (Table 4).

The medium rates of the oil-based ES and WP formulations of *B. bassiana* applied alone showed similar efficacy

Table 3
Effects of foliar spray applications of *B. bassiana*- and *B. thuringiensis tenebrionis*-based biopesticides against Colorado potato beetle larvae in a July 1999 field trial

Treatment[a]	Larvae[b]	Observed % control	Expected % control[c]	Synergism (+) or antagonism (−)	ANOVA main effects and interactions	
Controls	20.2 ± 1.33	—			Bt	$F_{[1,21]} = 298.3$; $P < 0.0001$
Bt-L	2.5 ± 0.35	87.6			Bb	$F_{[1,21]} = 16.6$; $P = 0.0004$
Bb-ES-M	17.4 ± 2.64	13.9			Bt × Bb	$F_{[1,21]} = 8.36$; $P = 0.0056$
Bt-L + Bb-ES-M	1.0 ± 0.23	95.1	89.3	+5.8 %		

[a] For complete description of treatments, see Table 1.
[b] Mean larvae per 10 stems per sample date from four consecutive samples collected 8, 10, 11, and 14 days after initial application (± standard error; controls, $n = 12$; treatments, $n = 6$).
[c] Control predicted if the agents exhibit independent action.

(days 9–16 $F_{[1,4]} = 0.5$; $P = 0.506$) and provided no significant control of beetle larvae (days 9–16 $F_{[1,14]} = 1.6$: $P = 0.226$ and $F_{[1,14]} = 1.3$; $P = 0.306$, respectively) (Figs. 3B and C). As seen in the 1998 and 1999 seasons, the combined medium rate of the fungal product and low rate of the bacterial product produced the greatest numerical reduction in larval populations (>90% control within 12 days). However, in these tests, the differences between populations treated with the combined bacterial and fungal products versus the bacterial product alone were not statistically significant. This was the case with both the ES and WP formulations (days 12–21 $F_{[1,4]} = 5.1$; $P = 0.086$ and $F_{[1,4]} = 5.9$; $P = 0.071$, respectively). The ANOVAs also revealed no significant interactions ($P = 0.164$ and 0.090, respectively, Table 4). The P values in these tests were low, however (in most cases significant at the $\alpha = 0.10$ level), and the ANOVA including all treatments did reveal a synergistic interaction (days 12–21 $F_{[1,42]} = 5.1$; $P = 0.030$; Table 4). There was an overall 11% greater reduction in larval numbers than predicted by independent action.

3.5. Damage assessments

Significant defoliation damage was recorded during the 1998 and 1999 seasons; however, defoliation at levels impacting yield were observed only in 1999 (Table 5). Addition of *Beauveria* to the *B. thuringiensis* treatment did not increase yield. No significant differences in yield were found among the various treatments applied in 2000 ($F_{[7,38]} = 0.4$, $P = 0.897$).

4. Discussion

The poor performance of the *B. bassiana* treatments was not unexpected. Previous researchers have modeled the *B. bassiana–L. decemlineata* biological control system and concluded that applications at 7-day intervals are inadequate to achieve effective larval control (Galaini, 1984); this was recently verified in field tests (Wraight and Ramos, 2002). Control with *Beauveria* alone was not our objective here. Interactions between the agents were explored by application of only two sprays at weekly intervals.

Our finding of a synergistic interaction between the *B. thuringiensis*- and *B. bassiana*-based biopesticides contrasts with the results of Sandner and Cichy (1967), Lewis and Bing (1991), and Costa et al. (2001). The reasons for this are not known; however, any comparative interpretation must take into account the many variables. The studies by Sandner and Cichy (1967) and Lewis and Bing (1991) were conducted with different strains of *B. bassiana* and a different subspecies of *B. thuringiensis*, and these microbes were applied against lepidopteran larvae. Moreover, the pathogens were applied in pure form or in formulations that differed substantially from those tested in our study, and only one of the studies (Lewis and Bing, 1991) was conducted in the field. More significantly, our results appear to disagree with the findings of a study by Costa et al. (2001) in which no synergism was observed between *B. t. tenebrionis* and *B. bassiana* strain GHA applied also against Colorado potato beetle larvae. However, this study differed even more substantially from ours in that the insects were not exposed to both pathogens simultaneously; cryIIIA δ-endotoxin was applied against early fourth-instar larvae, and the fungus was applied several days later against fully developed fourth-instars (prepupae) that survived intoxication.

In view of this, and considering the well-documented potential for various interactions among biopesticides, we do not consider our finding of synergism to be particularly surprising. Synergism has been reported between entomopathogenic fungi and other pathogens (Fuxa, 1979), including other species of entomopathogenic bacteria (Glare, 1994), and between pathogens other than fungi (see Krieg, 1971). Cases of synergism between fungal pathogens and toxic chemical insecticides were cited in the introduction, and the principal action of *B. thuringiensis tenebrionis* against potato beetle larvae is, in fact, toxinosis. In addition, it has been demonstrated that formulation ingredients widely used in modern biopesticide formulations may synergize the activity of entomopathogens (see Burges, 1998). Ultimately, the observed synergism might be explained by any of several mechanisms.

The synergistic interaction may have resulted from the straightforward effects of larval intoxication on rates of successful fungal penetration. It is well known that the molting process can remove infectious fungal inoculum from an insect and thus spare the host from infection (Vandenburg et al., 1998; Vey and Fargues, 1977). Further, it is

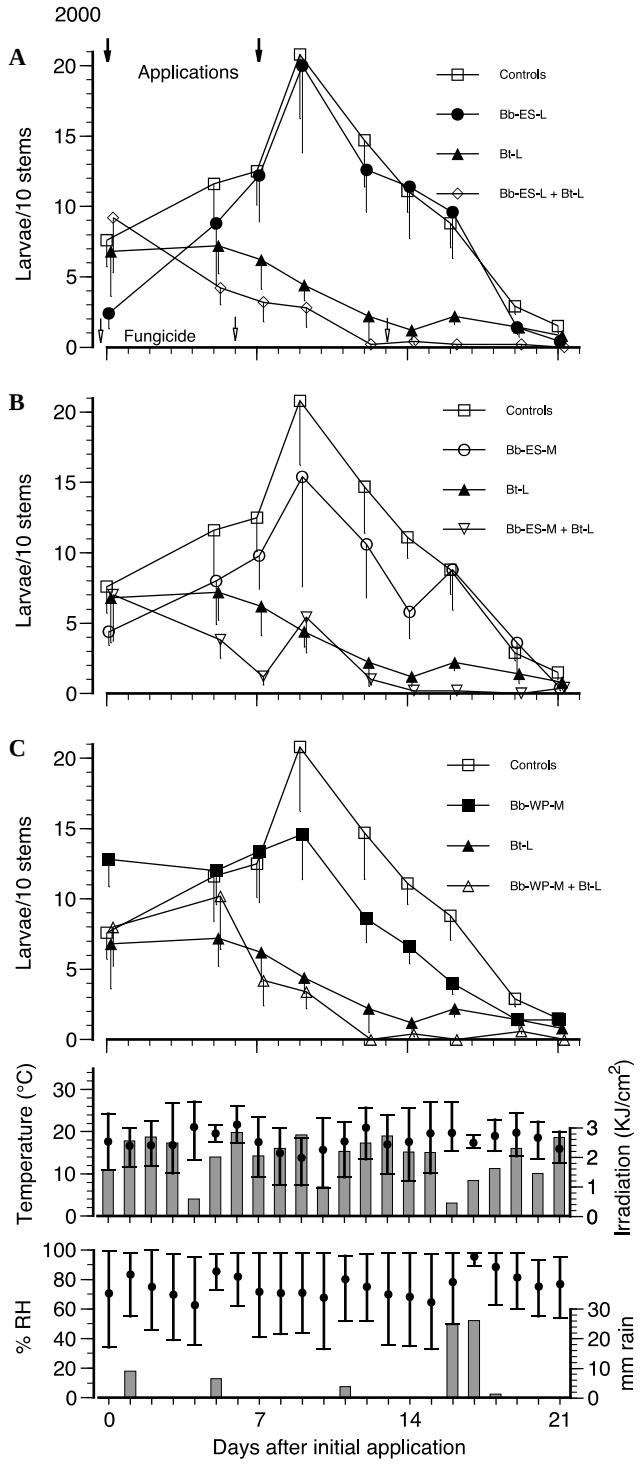

Fig. 3. Trends in larval Colorado potato beetle populations during a program of spray applications of *B. bassiana*- and *B. thuringiensis*-based biopesticides during a 2000 field trial with moisture and temperature data. Explanation of treatment codes in figure legend is presented in Table 1. Vertical lines represent standard errors of means; for clear presentation of standard errors, some means are offset on the *x* axis.

known that sublethal doses of *B. thuringiensis tenebrionis* retard development of potato beetle larvae (Cloutier and Jean, 1998; Costa et al., 2000). The hypothesis follows, therefore, that *B. thuringiensis* might synergize *B. bassiana* activity by prolonging the interval of time between molts (providing the fungus with more time to breach the cuticle before being cast off). A synergistic response would result as long as the physiology of the cuticle or hemolymph of intoxicated hosts was not altered in some way conferring resistance to fungal penetration or colonization.

This mode of action might explain the low and unpredictable level of synergism observed here. *B. bassiana* is highly pathogenic to Colorado potato beetle, and the spore germination and infection process is rapid under favorable environmental conditions (Fargues, 1972; Vey and Fargues, 1977; Inglis et al., 1996). The effect of a delayed molt, especially under optimal conditions, might therefore have only a small effect on ultimate levels of infection and mortality. The effect would also be dependent upon the timing of the application relative to the developmental stage of a larval cohort. The effect would be marginal if the treatments were applied optimally (at the beginning of a larval instar). It is interesting that the levels of synergism were greatest in the 1998 test in which the treatments were initiated against late instars. It is possible that the population was at a critical stage of development with respect to a positive interaction between *B. bassiana* and *B. thuringiensis*. Glare (1994) observed a synergistic response between the fungus *M. anisopliae* and the bacterial pathogen *Serratia entomophila* in second-instar larvae of a scarab beetle, but this interaction was not observed in third instars. The mechanism behind this response was not determined. The greater level of synergism might also be related to the fact that large larvae are less susceptible to *B. thuringiensis* (Zehnder and Gelernter, 1989). As a consequence, there may simply have been more sublethally affected larvae subjected to *B. bassiana* infection in 1998 than in 1999 or 2000.

Another mechanism of synergistic action between *B. bassiana* and *B. thuringiensis* is suggested by the recent studies of Furlong and Groden (2003). These researchers demonstrated that starvation markedly increased the susceptibility of early second-instar Colorado potato beetle larvae to *B. bassiana* infection. Starvation increases the inter-molt period (Furlong and Groden, 2003), and this was suspected as a primary mechanism of increased susceptibility. However, it was also found that the insect growth regulator Cyromazine increased inter-molt period without significantly affecting susceptibility to the fungus (Furlong and Groden, 2001), and it was ultimately concluded that some unknown effect of starvation on host physiology was responsible for the change in susceptibility. As intoxication by *B. thuringiensis* interrupts larval feeding, at least some level of starvation stress would result, and various effects on host physiology (other than, or in addition to, slowed development) could be critical factors in the synergistic interaction reported here. Furlong and Groden (2003) found susceptibility to be affected only by high levels of starvation stress (larvae deprived of food for 24 h). The percentage of the Freeville field populations that might have been affected to such a degree (without being killed outright by *B. thuringiensis* intoxication) is unknown; however, numerous

Table 4
Effects of foliar spray applications of *B. bassiana*- and *B. thuringiensis tenebrionis*-based biopesticides against Colorado potato beetle larvae in a July 2000 field trial

Treatments[a]	Larvae[b]	Observed % control	Expected[c] % control	Synergism (+) or antagonism (−)	ANOVA main effects and interactions	
Controls	7.8 ± 1.18	—			Bt	$F_{[1,22]} = 76.4$; $P < 0.001$
Bt-L	1.6 ± 0.69	79.5			Bb	$F_{[1,22]} = 6.9$; $P = 0.016$
Bb-ES-L	7.1 ± 1.94	9.0			Bt × Bb	$F_{[1,22]} = 5.2$; $P = 0.032$
Bt-L + Bb-ES-L	0.2 ± 0.06	97.4	81.3	+16.1 %		
Controls	7.8 ± 1.18	—			Bt	$F_{[1,22]} = 59.1$; $P < 0.0001$
Bt-L	1.6 ± 0.69	79.5			Bb	$F_{[1,22]} = 6.0$; $P = 0.023$
Bb-ES-M	5.8 ± 1.46	25.6			Bt × Bb	$F_{[1,22]} = 2.1$; $P = 0.164$
Bt-L + Bb-ES-M	0.4 ± 0.16	94.9	84.7	+10.2 %		
Controls	7.8 ± 1.18	—			Bt	$F_{[1,22]} = 89.8$; $P < 0.0001$
Bt-L	1.6 ± 0.69	79.5			Bb	$F_{[1,22]} = 16.6$; $P = 0.0005$
Bb-WP-M	4.4 ± 0.83	43.6			Bt × Bb	$F_{[1,22]} = 3.1$; $P = 0.090$
Bt-L + Bb-WP-M	0.2 ± 0.09	97.4	88.4	+9.0 %		
Controls	7.8 ± 1.18	—			Bt	$F_{[1,42]} = 127.1$; $P < 0.0001$
Bt-L	1.6 ± 0.69	79.5			Bb	$F_{[1,42]} = 13.4$; $P = 0.0007$
All Bb	5.6 ± 0.87	28.2			Bt × Bb	$F_{[1,42]} = 5.1$; $P = 0.030$
All Bt + Bb	0.3 ± 0.06	96.2	85.3	+10.9 %		

[a] For complete description of treatments, see Table 1.
[b] Mean larvae per 10 stems per sample date from five consecutive samples collected 12, 14, 16, 19, and 21 days after initial application (± standard error; controls, $n = 15$; individual treatments, $n = 5$; all Bb and all Bt + Bb, $n = 15$).
[c] Control predicted if the agents exhibit independent action.

Table 5
Colorado potato beetle larval populations and levels of damage in plots receiving various *B. bassiana* and/or *B. thuringiensis tenebrionis* treatments during two field tests in Freeville, New York

Treatment[a]	1998		1999			
	Defoliation (%)[b]	ANOVA statistics[c]	Defoliation (%)[b]	ANOVA statistics[c]	Yield (kg)	ANOVA statistics[c]
Controls	9.6 ± 1.3		32.0 ± 4.3		0.53 ± 0.08	
Bb-ES-L	12.1 ± 1.2	—	—	—	—	—
Bb-ES-M	8.2 ± 1.0	$q = 0.61$; $P > 0.05$	19.8 ± 4.9	$q = 2.36$; $P < 0.05$	0.62 ± 0.11	$q = 1.51$; $P > 0.05$
Bt-L	2.9 ± 1.1	$q = 4.76$; $P < 0.01$	5.8 ± 0.5	$q = 5.63$; $P < 0.01$	0.70 ± 0.12	$q = 3.7$; $P < 0.01$
Bt-H	2.5 ± 1.1	$q = 5.42$; $P < 0.01$	—	—	—	—
Bt-L + Bb-ES-L	4.7 ± 1.4	$q = 3.60$; $P < 0.01$	—	—	—	—
Bt-L + Bb-ES-M	2.9 ± 1.1	$q = 5.10$; $P < 0.01$	4.8 ± 0.6	$q = 6.0$; $P < 0.01$	0.62 ± 0.10	$q = 1.83$; $P > 0.05$
Bt-H + Bb-ES-M	1.0 ± 0.07	$q = 7.73$; $P < 0.01$	—	—	—	—

[a] For complete description of treatments, see Table 1.
[b] Percent defoliation (± standard error; 1998 combined controls, $n = 15$; treatments, $n = 5$; 1999 combined controls, $n = 12$, treatments, $n = 6$).
[c] Dunnett's q test (one-tailed hypothesis; 1998 df = 33, 8; 1999 df = 21, 4) comparing each fungal treatment to the combined controls.

researchers have reported severe to nearly complete disruption of feeding for 12–48 h after exposure of potato beetle larvae to sublethal doses of *B. thuringiensis* in laboratory tests (Cloutier and Jean, 1998; Costa et al., 2000; Hough-Goldstein et al., 1991; Zehnder and Gelernter, 1989).

Alternatively, enhancement of *B. thuringiensis* efficacy by *B. bassiana* is also possible. Fargues et al. (1994) observed a 20% increase in Colorado potato beetle larval feeding (phagostimulation) during the initial 24 h of exposure to *B. bassiana* conidia. Any increase in feeding would lead to greater consumption of bacterial toxin associated with treated foliage. This might be especially important under environmental conditions unfavorable to toxin persistence on potato foliage.

Finally, it is possible that the synergism we observed between these commercial biopesticides was produced not by any direct effects of the pathogens on the host or on one another, but, rather, due to effects of the formulation ingredients in the biopesticides. Our field tests did not include all possible pathogen-carrier blank treatment combinations (we did not test the fungal product in combination with the bacterial product formulation blank nor the bacterial product in combination with the two fungal product formulation blanks). The potential of various commercial biopesticide formulation ingredients to enhance efficacy of insect pathogens was noted in the preceding discussion. It has been demonstrated, for example, that surfactants can enhance the insecticidal activity of *B. thuringiensis* (Morris et al., 1995; Salama et al., 1985). Salama et al. (1985) hypothesized that surfactants disrupt the insect gut epithelium, increasing permeability to bacterial toxins. The *B. bassiana* formulations used in this study contain powerful wetting agents and emulsifiers, and these materials may have affected the efficacy of *B. thuringiensis*. Similarly,

materials in the *B. thuringiensis* formulation may have affected the efficacy of *B. bassiana*. Our tests also did not include any combinations of the formulation blanks alone, and the possibility exists of interactions among the formulation ingredients. These possibilities warrant additional study; although, such investigations could be conducted more efficiently in the laboratory or greenhouse.

Despite the unknown effects of the many proprietary formulation ingredients, we consider the findings of practical value because formulation materials are integral parts of nearly all biopesticides, and bacterial and fungal products formulated for aqueous spray applications generally contain ingredients similar to those used in the Mycotrol and Novodor products. It is impractical, for example, to apply the extremely hydrophobic conidia of *B. bassiana* in aqueous sprays without formulation with strong surfactants.

Potential effects of the various chemical pesticides (primarily fungicides) on the host–pathogen-formulation ingredient interactions must also be considered. The pesticide applications in our research plots were mandated by needs to control pests and diseases that threatened our crops and those of neighboring studies (see Section 2.2). Potato disease management is highly weather dependent and requires rotation of fungicides, so the applications also were not consistent across field seasons. However, all of these materials were applied uniformly over all plots (treatments and controls) within each field season. During the exceptionally dry year of 1999, only a single fungicide application was made, and this was after the biopesticide treatment effects were observed. A chemical insecticide was applied on just one occasion (for leafhopper control in 1998). Despite the great variability of the chemical pesticide applications from year to year, synergism between the biopesticides was a consistent finding.

The importance of the observed low-level synergism with respect to pest control and crop production is difficult to assess. Potatoes can tolerate a considerable amount of defoliation (Hare, 1980; Zehnder et al., 1995), and the differences in yields resulting from addition of *B. bassiana* to *B. thuringiensis* applications would probably be small and difficult to detect employing standard experimental field-test designs. In the tests reported herein, beetle populations were so low, or plants were so severely stressed from drought conditions, that few differences were observed in yields from the various treatments and controls. Nevertheless, the observation of synergism between the low doses of both pathogens (Fig. 3A and Table 4) is noteworthy. Additional study is needed to confirm that low rates of both pathogens might be synergistic (observed here in only one of two tests). Microbial insecticides are generally more costly to apply than chemical insecticides, and any strategies allowing for reductions in rate are important in economic terms.

The results are most significant in demonstrating an exceptionally high level of compatibility between these two biological control agents. As discussed in the introduction, *B. bassiana* generally acts too slowly to protect potatoes from significant defoliation. However, mycosis may ultimately be expressed at high levels in the soil after larvae begin the process of pupation (Fargues, 1972; Lacey et al., 1999; Roberts et al., 1981; Timonin, 1939; Wraight and Ramos, 2002). The fungus can also persist in the soil and have significant long-term impacts on beetle populations (Watt and LeBrun, 1984; Wojciechowska et al., 1977). These characteristics make combination with the fast-acting, short-residual *B. thuringiensis* an attractive approach. The *B. thuringiensis* component would provide rapid control and foliage protection, while the *B. bassiana* component would contribute long-term suppression of subsequent generations (through persistent activity against beetle prepupae, pupae, and adults in the soil). A bio-intensive potato pest management system developed by Gallandt et al. (1998) that included combined use of *B. thuringiensis* and *B. bassiana* was demonstrated to provide excellent control of Colorado potato beetle over a 6-year period. It is not possible to determine the specific impacts of the microbial biocontrol components in this system, as the control strategy also included spray applications of rotenone and releases of large numbers of the predatory bug *Perillus bioculatus*. Cloutier and Jean (1998) demonstrated that *B. thuringiensis* could synergize the predatory activities of *P. bioculatus*. The bio-based system of Gallandt et al. (1998) also proved more costly than conventional management practices, but the study nevertheless demonstrated considerable potential for use of *B. bassiana* and *B. thuringiensis* as key elements of a multi-component integrated pest management system for potato beetle management. Clearly, programs such as this have great potential as well to reduce selection pressures and development of resistance.

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References

Anderson, T.E., Hajek, A.E., Roberts, D.W., Preisler, H.K., Robertson, J.L., 1989. Colorado potato beetle (Coleoptera: Chrysomelidae): effects of combinations of *Beauveria bassiana* with insecticides. J. Econ. Entomol. 82, 83–89.

Benz, G., 1971. Synergism of micro-organisms and chemical insecticides. In: Burges, H.D., Hussey, N.W. (Eds.), Microbial Control of Insects and Mites. Academic Press, London, pp. 327–355.

Bradley, C.A., Black, W.E., Kearns, R., Wood, P., 1992. Role of production technology in mycoinsecticide development. In: Leatham, G.F. (Ed.), Frontiers in Industrial Mycology. Chapman & Hall, New York, pp. 160–173.

Bradley, C.A., Wood, P.P., Britton, J., 2001. Mycoinsecticides against an insect of the grasshopper family. United States Patent No. 6,261,553.

Burges, H.D. (Ed.), 1998. Formulation of Microbial Biopesticides. Kluwer Academic Publishers, London.

Cloutier, C., Jean, C., Bauduin, F., 1995. More biological control for a sustainable potato pest management strategy. In: Duchesne, R.-M., Boiteau, G. (Eds.), Potato Insect Pest Control. Agriculture and Agri-Food Canada, Quebec, pp. 15–52.

Cloutier, C., Jean, C., 1998. Synergism between natural enemies and biopesticides: a test case using the stinkbug *Perillus bioculatus* (Hemiptera: Pentatomidae) and *Bacillus thuringiensis tenebrionis* against Colorado potato beetle (Coleoptera: Chrysomelidae). J. Econ. Entomol. 91, 1096–1108.

Costa, S.D., Barbercheck, M.E., Kennedy, G.G., 2000. Sublethal acute and chronic exposure of Colorado potato beetle (Coleoptera: Chrysomelidae) to the delta-endotoxin of *Bacillus thuringiensis*. J. Econ. Entomol. 93, 680–689.

Costa, S.D., Barbercheck, M.E., Kennedy, G.G., 2001. Mortality of Colorado potato beetle (*Leptinotarsa decemlineata*) after sublethal stress with the CRYIIIA delta-endotoxin of *Bacillus thuringiensis* and subsequent exposure to *Beauveria bassiana*. J. Invertebr. Pathol. 77, 173–179.

Deacon, J.W., 1983. Microbial Control of Plant Pests and Diseases. Van Nostrand Reinhold Co, Wokingham, UK.

Fargues, J., 1972. Étude des conditions d'infection des larves de doryphore, *Leptinotarsa decemlineata* Say, par *Beauveria bassiana* (Bals.) Vuill. (Fungi Imperfecti). Entomophaga 17, 319–337.

Fargues, J., Delmas, J.C., LeBrun, R.A., 1994. Leaf consumption by larvae of the Colorado potato beetle (Coleoptera: Chrysomelidae) infected with the entomopathogen *Beauveria bassiana*. J. Econ. Entomol. 87, 67–71.

Ferron, P., 1985. Fungal control. In: Kerkut, G.A., Gilbert, L.I. (Eds.), Comprehensive Insect Physiology Biochemistry and Pharmacology. Vol. 12. Insect Control. Pergamon Press, Oxford, pp. 313–346.

Forgash, A.J., 1981. Insecticide resistance of the Colorado potato beetle, *Leptinotarsa decemlineata* (Say). In: Lashomb, J.H., Casagrande, R. (Eds.), Advances in Potato Pest Management. Hutchinson Ross Publishing Co, Stroudsburg, PA, pp. 34–46.

Furlong, M.J., Groden, E., 2001. Evaluation of synergistic interactions between the Colorado potato beetle (Coleoptera: Chrysomelidae) pathogen *Beauveria bassiana* and the insecticides imidacloprid and cyromazine. J. Econ. Entomol. 94, 344–356.

Furlong, M.J., Groden, E., 2003. Starvation induced stress and the susceptibility of the Colorado potato beetle, *Leptinotarsa decemlineata*, to infection by *Beauveria bassiana*. J. Invertebr. Pathol. 83, 127–138.

Fuxa, J.R., 1979. Interactions of the microsporidium *Vairimorpha necatrix* with a bacterium, virus, and fungus in *Heliothis zea*. J. Invertebr. Pathol. 33, 316–323.

Galaini, S., 1984. The efficacy of foliar applications of *Beauveria bassiana* conidia against *Leptinotarsa decemlineata*. MS Thesis. Cornell Univerisity, Ithaca, NY, pp. 94.

Gallandt, E.R., Mallory, E.B., Alford, A.R., Drummond, F.A., Groden, E., Liebman, M., Marra, M.C., McBurnie, J.C., Porter, G.A., 1998. Comparision of alternative pest and soil management strategies for Maine potato production systems. Am. J. Altern. Agric. 13, 146–161.

Glare, T.R., 1994. Stage-dependent synergism using *Metarhizium anisopliae* and *Serratia entomophila* against *Costelytra zealandica*. Biocontrol Sci. Technol. 4, 321–329.

Hajek, A.E., Soper, R.S., Roberts, D.W., Anderson, T.E., Biever, K.D., Ferro, D.N., LeBrun, R.A., Storch, R.H., 1987. Foliar applications of *Beauveria bassiana* (Balsamo) Vuillemin for control of the Colorado potato beetle, *Leptinotarsa decemlineata* (Say) (Coleoptera: Chrysomelidae): an overview of pilot test results from the northern United States. Can. Entomol. 119, 959–974.

Hare, J.D., 1980. Impact of defoliation by the Colorado potato beetle on potato yields. J. Econ. Entomol. 73, 369–373.

Hough-Goldstein, J., Tisler, A.M., Zehnder, G.W., Uyeda, K.A., 1991. Colorado potato beetle (Coleoptera: Chrysomelidae) consumption of foliage treated with *Bacillus thuringiensis* var. *san diego* and various feeding stimulants. J. Econ. Entomol. 84, 87–93.

Humber, R.A., 1996. Fungal pathogens of the Chrysomelidae and prospects for their use in biological control. In: Jolivet, P.H.A., Cox, M.L. (Eds.), Chrysomelidae Biology, vol. 2: Ecological Studies. SPB Academic Publishing, Amsterdam, pp. 93–115.

Inglis, G.D., Johnson, D.L., Goettel, M.S., 1996. Effects of temperature and thermoregulation on mycosis by *Beauveria bassiana* in grasshoppers. Biol. Control 7, 131–139.

Jaques, R.P., Laing, D.R., 1989. Effectiveness of microbial and chemical insecticides in control of the Colorado potato beetle (Coleoptera: Chrysomelidae) on potatoes and tomatoes. Can. Entomol. 120, 1123–1131.

Jaworska, M., 1987. Combined application of some insecticidal fungi and insecticides against *Acanthoscelides obtectus* Say and *Leptinotarsa decemlineata* Say. Pol. Ecol. Stud. 13, 231–265.

Krieg, A., 1971. Interactions between pathogens. In: Burges, H.D., Hussey, N.W. (Eds.), Microbial Control of Insects and Mites. Academic Press, London, pp. 459–468.

Krieg, A., Huger, A.M., Langenbruch, G.A., Schnetter, W., 1983. *Bacillus thuringiensis* var. *tenebrionis*: Ein neuer, gegenüber larven von coleopteren wirksamer pathotyp. Z. Angew. Entomol. 96, 500–508.

Lacey, L.A., Horton, D.R., Chauvin, R.L., Stocker, J.M., 1999. Comparative efficacy of *Beauveria bassiana*, *Bacillus thuringiensis*, and aldicarb for control of Colorado potato beetle in an irrigated desert agroecosystem and their effects on biodiversity. Entomol. Exp. Appl. 93, 189–200.

Lewis, L.C., Bing, L.A., 1991. *Bacillus thuringiensis* Berliner and *Beauveria bassiana* (Balsamo) Vuillemin for European corn borer control: program for immediate and season-long suppression. Can. Entomol. 123, 387–393.

Lipa, J.J., 1985. Progress in biological control of the Colorado beetle (*Leptinotarsa decemlineata*) in eastern Europe. Bull. OEPP 15, 207–211.

Morris, O.N., Converse, V., Kanagaratnam, P., 1995. Chemical additive effects on the efficacy of *Bacillus thuringiensis* Berliner subsp. *kurstaki* against *Mamestra configurata* (Lepidoptera: Noctuidae). J. Econ. Entomol. 88, 815–824.

Navon, A., 2000. *Bacillus thuringiensis* insecticides in crop protection—reality and prospects. Crop Protect. 19, 669–676.

Poprawski, T.J., Carruthers, R.I., Speese III, J., Vacek, D.C., Wendel, L.E., 1997. Early-season applications of the fungus *Beauveria bassiana* and introduction of the Hemipteran Predator *Perillus bioculatus* for control of Colorado potato beetle. Biol. Control 10, 48–57.

Roberts, D.W., LeBrun, R.A., Semel, M., 1981. Control of the Colorado potato beetle with fungi. In: Lashomb, J.H., Casagrande, R. (Eds.), Advances in Potato Pest Management. Hutchinson Ross Publishing Company, Stroudsburg, pp. 119–137.

Salama, H.S., Foda, M.S., Sharaby, A., 1985. Potential of some chemicals to increase the effectiveness of *Bacillus thuringiensis* Berl. against *Spodoptera littoralis* (Boisd). J. Appl. Entomol. 100, 425–433.

Sandner, H., Cichy, D., 1967. Research on the effectiveness of fungal and bacterial insecticides. Ekol. Pol. Ser. A 15, 325–333.

SAS Institute, 1995. JMP® Statistics and Graphics Guide: Version 3.1. SAS Institute Inc., Cary, NC.

Sokal, R.R., Rohlf, F.J., 1995. Biometry. W.H. Freeman and Co, San Francisco.

Storch, R.H., 1995. Insect control on potato with conventional insecticides. In: Duchesne, R.-M., Boiteau, G. (Eds.), Potato Insect Pest Control. Agriculture and Agri-Food Canada, Quebec, pp. 95–112.

Telenga, N.A., 1962. Le probleme de l'utilisation des micro-organismes entomopathogenes en combinaison avec les insecticides. Entomophaga Mem. Hors Ser. 2, 531–544.

Timonin, M.I., 1939. Pathogenicity of *Beauveria bassiana* (Bals.) Vuill. on Colorado potato beetle larvae. Can. J. Res. 17, 103–107.

Vandenburg, J.D., Ramos, M., Altre, J.A., 1998. Dose-response and age- and temperature-related susceptibility of the diamondback moth (Lepidoptera: Plutellidae) to two isolates of *Beauveria bassiana* (Hyphomycetes: Moniliaceae). Environ. Entomol. 27, 1017–1021.

Vey, A., Fargues, J., 1977. Histological and ultrastructural studies of *Beauveria bassiana* infection in *Leptinotarsa decemlineata* larvae during ecdysis. J. Invertebr. Pathol. 30, 207–215.

Watt, B.A., LeBrun, R.A., 1984. Soil effects of *Beauveria bassiana* on pupal populations of the Colorado potato beetle (Coleoptera: Chrysomelidae). Environ. Entomol. 13, 15–18.

Wojciechowska, M., Kmitowa, K., Fedorko, A., Bajan, C., 1977. Duration of activity of entomopathogenic organisms introduced into the soil. Pol. Ecol. Stud. 3, 141–148.

Wraight, S.P., Carruthers, R.I., 1999. Production, delivery, and use of mycoinsecticides for control of insect pests of field crops. In: Hall, F.R., Menn, J.J. (Eds.), Methods in Biotechnology, Vol. 5: Biopesticides: Use and Delivery. Humana Press, Totowa, NJ, pp. 233–269.

Wraight, S.P., Ramos, M.E., 2002. Application parameters affecting field efficacy of *Beauveria bassiana* foliar treatments against Colorado potato beetle *Leptinotarsa decemlineata*. Biol. Control 23, 164–178.

Zehnder, G.W., Gelernter, W.D., 1989. Activity of the M-ONE formulation of a new strain of *Bacillus thuringiensis* against the Colorado potato beetle (Coleoptera: Chrysomelidae): relationship between susceptibility and insect life stage. J. Econ. Entomol. 82, 756–761.

Zehnder, G., Vencill, A.M., Speese III, J., 1995. Action thresholds based on plant defoliation for management of Colorado potato beetle (Coleoptera: Chrysomelidae) in potato. J. Econ. Entomol. 88, 155–161.