Assessment of *Beauveria bassiana* Sprays for Control of Diamondback Moth (Lepidoptera: Plutellidae) on Crucifers

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ABSTRACT Evaluations of the efficacy and persistence of *Beauveria bassiana* (Balsamo) Vuillemin for control of the diamondback moth, *Plutella xylostella* (L.),on crucifers were done in growth chambers, the greenhouse, and the field. In growth chamber studies done at 21 or 26°C and 60 or 90% RH, neither temperature nor humidity affected the survival of larvae, but treatment with fungal spores always provided significantly greater mortality than the control. In 2 greenhouse trials, 1 application of *B. bassiana* spores suspended in water or oil significantly reduced larval populations compared to controls. In a field trial, both treatment and insect stage at treatment (2nd and 3rd to 4th instars) significantly affected larval survival. The fungus, formulated as a wettable powder at 2 rates and as an emulsifiable suspension at a high rate, provided significant reductions in larval counts. Two applications of the wettable powder at the higher rate resulted in lower larval counts than did a single application. Two applications (but not 1) of wettable powder at the lower rate resulted in significantly lower counts of 2nd instars but not 3rd to 4th instars. Both treatment and time affected both the persistence of viable *B. bassiana* spores on leaves and the retention of efficacy of treated leaves for *P. xylostella* larvae. These trials indicate the potential for including *B. bassiana* in an overall management program for *P. xylostella*.

KEY WORDS *Plutella xylostella*, insecticide resistance, crucifers, microbial control, insect pathogen

THE DIAMONDBACK MOTH, *Plutella xylostella* (L.), is considered the most important insect pest of crucifers worldwide with an estimated annual cost of control of $1 billion (Talekar and Shelton 1993). This insect has been especially problematic because populations in several parts of the world have developed resistance to most commercial insecticides, including some growth regulators and toxins contained in at least 2 subspecies of the microbial insecticide *Bacillus thuringiensis* Berliner (Perez et al. 1997). Natural enemies are fundamental for an integrated and sustainable management program for control of *P. xylostella*, but supplemental control through the use of a foliar-applied insecticide is necessary when an economic threshold is exceeded (Shelton et al. 1983). Because of *P. xylostella* resistance to many of the commercially available insecticides (Shelton et al. 1993a, b), it is important to search for other potentially useful insecticides.

Foliar applications of *Beauveria bassiana* (Balsamo) Vuillemin have proven to be useful in suppressing populations of several economically important insects, including *Bemisia tabaci* (Gennadius) (Wright 1992, Carruthers et al. 1993), *Diuraphis noxia* (Mordvilko) (J.D.V., unpublished data), *Leptinotarsa decemlineata* (Say) (Poprawski et al. 1997), *Ostrinia nubilalis* (Hübner) (Lewis et al. 1996), and *Franklinealla occidentalis* (Pergande) (S. Jaronski, personal communication) and may provide another important option for control of *P. xylostella* and other pests of crucifers. Several fungi have been isolated from *P. xylostella* (Humber 1992), and natural epizootics of 2 Entomophthorales species in Asian populations of *P. xylostella* have been described (Riethmacher and Kranz 1994). Pell et al. (1993) have studied infections of *Zoophthora radicans* (Brefeld) Batko, and Ibrahim and Low (1993) have shown the potential effectiveness of *B. bassiana* and *Paecilomyces fumosoroseus* (Wize) Brown & Smith in the field.

We have been working to incorporate fungi into management schemes for *P. xylostella*. In laboratory assays we have screened more than 55 isolates of *B. bassiana*, *Fusarium* sp., *Metarhizium anisopliae* (Metschnikoff) Sorokin and *Paecilomyces farinosus* (Holm ex S.F. Gray) Brown & Smith (Vandenberg and Ramos 1997) and determined the dose response and age- and temperature-related susceptibility of *P. xylostella* larvae to 2 *B. bassiana* isolates (Vandenberg et al. 1998). Based on these laboratory assays we evaluated foliar applications of *B. bassiana* as a strategy for protecting crucifer transplants against contamination by *P. xylostella* (Shelton et al. 1998). Previous studies have indicated that *P. xylostella*-contaminated transplants are a major problem in the overall strategy for managing *P. xylostella*. (Shelton et al. 1996)

Based on our previous laboratory assays and tests for control of *P. xylostella* on transplants, we believe there

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is considerable potential for the use of fungi for *P. xylostella* management under a broad range of conditions. The objectives of this study were to evaluate the efficacy of *B. bassiana* sprays against *P. xylostella* on whole plants, compare efficacies under varying abiotic conditions in growth chambers, evaluate alternative formulations in the greenhouse, and evaluate commercial formulations and alternative rates in the field.

Materials and Methods

Fungi. Fungal cultures of *B. bassiana* strain GHA (Mycotech, Butte, MT) and *B. bassiana* strain 4543 (USDA–ARS, Ithaca, NY) were maintained by transferring biweekly on Sabouraud's dextrose agar supplemented with 2% yeast extract (SDAY, DIFCO, Detroit) at 25° C and a photoperiod of 15:9 (L:D) h. For growth chamber and greenhouse tests, conidia were harvested by scraping from culture surfaces in petri dishes. For suspension treatments, spores were suspended in 0.1 % Tween 80 or 0.04% Silwet L-77 (Loveland, Greeley, CO) by mixing for 2 min on a vortexer (Scientific, Bohemia, NY). For emulsion treatments, spores were suspended in Mycotech Oil (Mycotech) by mixing for 2 min on a vortexer and emulsified at 1% in 0.1% (aqueous) Tween 80 with additional mixing. Concentrations were estimated with a hemacytometer and adjusted with additional 0.1% Tween 80 or 0.04% Silwet L-77. For field treatments, wettable powder (WP) and emulsifiable suspension (ES) of Mycotrol (Mycotech) were prepared in 0.04% Silwet L-77. Treatments were applied with a CO2-pressurized backpack sprayer (HC 12, R & D Sprayers, Opelousas, LA) with 3 single hollow cone nozzles (1 nozzle directed downward and 1 nozzle from each side) delivering 280.5 liters/ha at 2.8 kg/cm^2 at 3.2 kph.

Insects. In all trials plants were infested with *P. xylostella* from the Geneva 88 colony (Shelton et al. 1993a). This colony has been reared continuously in the laboratory and has been used as a standard susceptible colony for insecticide assays (Shelton et al. 1993a).

Growth Chamber. Broccoli (Green Goliath) seedlings were transplanted into 10-cm-diameter plastic pots containing field soil (Honeyoye Silt Loam) and maintained in the greenhouse at 25 ± 5°C with a photoperiod of 16:8 (L:D) h. Plants were moved outside to a grassy area when each plant had 5–6 true leaves (≈10 w after planting) and arranged with 45-cm plant spacing and 90-cm row spacing (field spacing). Plants were treated with *B. bassiana* strain GHA suspension at a rate of 5×10^{13} spores per hectare. Two hours after application of the fungus, each plant was infested with ten 2nd instars and placed in a growth chamber. Plants were randomly assigned to 1 of 4 incubation conditions: either 21 or 26°C and either 60 or 90% RH. Each treatment was replicated 3 times with 2 plants per replicate. All insects were counted 7 d after treatment.

Greenhouse. Broccoli (Green Goliath) seedlings were transplanted into 10-cm diameter plastic pots containing Metromix 360 (Sierra Horticultural, Marysville, OH) and infested at the 5- to 6-leaf stage. An aluminum strip containing ≈100 eggs was pinned to the underside of 1 leaf on each plant. When 2nd instars were visible on the plants, pots were arrayed outside (as previously described) and treated. In experiment 1, treatments included emulsified suspensions of *B. bassiana* strains 4543 and GHA applied at a rate of 5×10^{13} spores per hectare, an emulsion control, and a water-treated control. Larvae found alive at the end of the experiment (7 d) were maintained on untreated cabbage foliage in 10-cm petri dishes and monitored daily for 4 d. Dead larvae were placed on moistened filter paper in 6-cm petri dishes and monitored for appearance of *B. bassiana* hyphae and spores. In experiment 2, treatments included *B. bassiana* strain GHA suspension at a rate of 5×10^{13} spores per hectare, a suspension-only control, and an untreated control. In both experiments, each treatment was replicated 4 times, with 3 plants per replicate. Following treatment, plants were returned to the greenhouse and incubated in cages. Plants were destructively harvested, and all insects were counted 7 d after treatment.

Field Efficacy and Persistence. Cabbage ('Vantage Point') seedlings were transplanted to field plots on 15 June 1995 and maintained to the 10–12 leaf stage. Plots contained two 3.5-m rows of 7 plants per row at the previously described spacing. Three plants per plot were infested by pinning an aluminum strip containing ≈100 eggs to the underside of 1 leaf on each plant. Three additional plants per plot were infested by transferring 20 2nd instars from a laboratory colony to each plant. Applications were made when larvae reached the 2nd instar (for plants infested with eggs) and the 3rd to 4th instar (for plants infested with 2nd instars). Nine treatments included 2 rates of Mycotrol wettable powder (2.5 and 5×10^{13} spores per hectare) applied either once or twice (3-d interval), Mycotrol emulsifiable suspension at 1.4×10^{14} spores per hectare applied once, permethrin (Ambush 2 EC [emulsifiable concentrate], Zeneca, Wilmington, DE) at 0.11 kg (AI) / ha applied once, wettable powder carrier only applied twice, emulsifiable suspension carrier only applied once, and an untreated control. One week after the final applications, all insects were counted on all treated plants.

Leaf disc samples (2 cm diameter) were taken from leaves of plants in fungus-treated and untreated plots 1 h after treatment and 3 and 6 d later. Four discs, 1 from each of 4 plants from a single plot were mixed for 2 min in 10 ml sterile 0.1% Tween 80 using a vortexer. Samples of 0.1 ml were spread onto each of 3 SDAY plates and incubated for 3 d at 25°C and a photoperiod of 15:9 (L:D) h before counting all *B. bassiana* colonies.

Larger leaf disc samples (15 cm diameter) were cut from plants in fungus-treated and untreated plots immediately after treatment and at 3 and 6 d after treatment. Discs were placed individually in 15-cm petri dishes. Fifteen laboratory-reared 2nd instars were added to each dish and incubated at 25°C and a photoperiod of 15:9 (L:D) h. Survival was monitored daily

Table 1. Efficacy of *B. bassiana* strain GHA against *P. xylostella* larvae on broccoli in growth chambers

Treatment[a]	% RH	Temp, °C	Live larvae[b]
Suspension without fungus	60	21	6.3 ± 0.3a
		26	6.0 ± 0.8a
	90	21	7.7 ± 0.8a
		26	6.3 ± 0.7a
Suspension of *B. bassiana* strain GHA	60	21	4.2 ± 0.4b
		26	4.3 ± 0.2b
	90	21	2.0 ± 0.9b
		26	4.3 ± 1.9b

[a] Volume rate, 280 liters/ha; suspending agent, 0.1% Tween 80; *B. bassiana* rate, 5×10^{13} spores per hectare.

[b] Average number of live larvae per plant ± SEM. Larval counts were square-root transformed before ANOVA. Means within each experiment followed by the same letter are not significantly different by the Tukey-Kramer HSD test (Wilkinson et al. 1996), $P < 0.05$.

for 7 d. Dead larvae were removed to separate dishes with moistened filter paper and incubated for 1 d. Death caused by mycosis was diagnosed when *B. bassiana* hyphae and spores were evident.

Statistical Analyses. Analysis of variance (ANOVA) on square-root transformed counts of live larvae per replicate was used to determine treatment effects in growth chamber, greenhouse, and field tests. ANOVA treatment means were compared using the Tukey-Kramer honestly significant difference (HSD) test for growth chamber and greenhouse trials (Wilkinson et al. 1996) and Dunnet's test (to compare treatment means with a control) for field trials (Wilkinson et al. 1996). ANOVA on $\log_{10}$ number of colony-forming units (CFUs) per square millimeter of leaf surface was used to determine effects of treatment and time on spore survival in the field trial. The $\log_{10}$ CFUs per square millimeter of leaf surface was also regressed over time in days for each of 2 wettable powder treatments. ANOVA on angular-transformed percentage mortality was used to determine effects of treatment and time on the persistence of efficacy against *P. xylostella* larvae. Angular-transformed percentage mortality was regressed over time in days for each of the 3 fungal treatments in the field trial. All analyses were done using SYSTAT version 6.0 (SYSTAT 1996).

Results and Discussion

Growth Chamber. Fungus treatment significantly ($F = 20.3$; df = 1, 16; $P < 0.0001$) affected survival of *P. xylostella* larvae on plants in growth chambers (Table 1). However, abiotic incubation conditions did not affect survival (temperature: $F = 0.4$; df = 1, 16; $P < 0.55$; humidity: $F < 0.6$; df = 1, 16; $P < 0.45$), nor were there any significant interactions among treatment conditions. Vandenberg et al. (1998) showed that *B. bassiana* efficacy for *P. xylostella* was affected by temperature but found no differences among treatments at 20, 25 and 30°C. Although we used 2 different ambient relative humidities in this study, conditions near the leaf and insect surfaces may have been similar. Additional tests are needed to clarify the possible impact of relative humidity near fungal spores on field performance of *B. bassiana*.

Table 2. Efficacy of *B. bassiana* against *P. xylostella* larvae on broccoli in a greenhouse

Experiment	Treatment[a]	Live larvae[b]
1	Water	16.1 ± 2.8a
	Emulsion without fungus	14.3 ± 2.5a
	Emulsion of *B. bassiana* strain 4543	4.7 ± 1.3b
	Emulsion of *B. bassiana* strain GHA	5.9 ± 1.2b
2	Untreated	15.2 ± 1.2a
	Suspension without fungus	15.2 ± 1.2a
	Suspension of *B. bassiana* strain GHA	6.0 ± 1.5b

[a] Volume rate for all treatments, 280 liters/ha; suspending agent for all treatments, 0.1% Tween 80; emulsifying agent for experiment 1, Mycotech oil; *B. bassiana* rate for all treatments, 5×10^{13} spores per hectare.

[b] Average number of live larvae ± SEM per replicate consisting of 3 plants. Larval counts were square-root transformed before ANOVA. Means within each experiment followed by the same letter are not significantly different by the Tukey-Kramer HSD test (Wilkinson et al. 1996), $P < 0.05$.

Greenhouse. In the 1st greenhouse experiment, treatment with a *B. bassiana* emulsion resulted in significantly ($F = 10.1$; df = 3, 44; $P < 0.0001$) lower numbers of *P. xylostella* larvae compared to controls (Table 2). The percentages (and numbers) of larvae that died from mycosis following removal from treated plants, and maintenance on untreated foliage, were 0.6% ($n = 162$), 0% ($n = 158$), 70% ($n = 56$), and 54% ($n = 71$) for water-treated, emulsion-only treated, *B. bassiana* strain 4543 and *B. bassiana* strain GHA, respectively. This indicates the potential of continued mortality after a single application. Treatment with *B. bassiana* suspensions made from either of 2 fungus strains also signficantly ($F = 14.3$; df = 1, 8; $P < 0.005$) reduced *P. xylostella* numbers in the 2nd experiment (Table 2).

Field Efficacy and Persistence. Both treatment and insect stage at treatment, but not the interaction between them, significantly affected larval survival in the field (treatments: $F = 6.0$; df = 8, 54; $P < 0.001$; stage: $F = 21.2$; df = 1, 54; $P < 0.0001$; interaction: $F = 0.8$; df = 8, 54; $P < 0.6$). Because the insect stage at treatment significantly influenced fungal effectiveness, separate analyses were done for each.

Treatment significantly ($F = 4.7$; df = 8, 27; $P < 0.001$) affected survival of *P. xylostella* treated as 2nd instars (Table 3). All fungus treatments significantly ($P < 0.05$) reduced the number of live larvae per replicate compared to untreated controls (Table 3), except for the single application of wettable powder at the lower rate ($P < 0.06$). Among fungus treatments, greatest reductions were obtained from wettable powder treatments at the higher rate and from the single application of emulsifiable suspension. Two applications (versus 1) of wettable powder at the higher rate resulted in lower larval counts. There was no difference between 1 and 2 applications of wettable powder at the lower rate. Lowest survival was obtained in plots treated once with Ambush 2 EC.

Treatment also significantly ($F = 2.5$; df = 8, 27; $P < 0.03$) affected survival of *P. xylostella* treated as 3rd to 4th instars (Table 4). Fungus treatments with wettable powder at the higher rate and with emulsifiable sus-

Table 3. Efficacy of *B. bassiana* strain GHA applied to cabbage infested with 2nd instar *P. xylostella*

Treatment	Rate ($\times 10^{13}$ spores/ha)	No. of applications	Live larvae[a]	$P <$[b]
Untreated	—	—	6.8 ± 1.2	1.0
WP carrier only	—	2	5.2 ± 1.6	0.5
ES carrier only	—	1	6.8 ± 0.9	0.5
Mycotrol WP	5.0	2	1.1 ± 0.6	0.02
Mycotrol WP	2.5	2	1.5 ± 0.6	0.03
Mycotrol WP	5.0	1	0.8 ± 0.2	0.01
Mycotrol WP	2.5	1	1.5 ± 0.4	0.06
Mycotrol ES	14.0	1	0.8 ± 0.3	0.01
Ambush 2 EC	(0.11 kg [AI]/ha)	1	0.1 ± 0.1	0.001

[a] Average number of live larvae ± SEM per sample of 3 plants per plot. Larval counts were square-root transformed before ANOVA.
[b] Significance of difference from untreated control by the Dunnet test (Wilkinson et al. 1996).

pension gave significant reductions in larval counts. Two applications (versus 1) of wettable powder at the higher rate resulted in lower larval counts. Neither 1 nor 2 applications of wettable powder at the lower rate resulted in significantly lower larval counts. Lowest survival was obtained in plots treated once with Ambush.

Both treatment and day affected survival of fungal spores on leaves (treatment: $F = 14.3$; df = 2, 30; $P < 0.0001$; day: $F = 111.8$; df = 2, 30; $P < 0.0001$). The number of viable fungal spores on foliage declined significantly over time following treatment at either rate of wettable powder. At the lower rate, a quadratic nonlinear regression was significant (Fig. 1a; $F = 135$; df = 3, 11; $P < 0.0001$; $\log_{10}$ CFUs/mm^2 = 2.18 − 0.75 [day] + 0.06 [day^2]; $r^2 = 0.95$; standard errors were 0.11, 0.09, and 0.02 for the 3 parameters, respectively). At the higher rate, a quadratic regression was also significant (Fig. 1b; $F = 509$; df = 3, 12; $P < 0.0001$; $\log_{10}$ CFUs/mm^2 = 2.40 - 0.82 [day] + 0.08 [day^2]; $r^2 = 0.97$; SEs were 0.06, 0.06, and 0.01 for the 3 parameters, respectively). For the emulsifiable suspension treatment, foliage samples taken at 6 d became contaminated with bacteria so regression of mortality over time was not possible. The average number of $\log_{10}$ CFUs recovered from emulsifiable suspension-treated leaves at day 0 and day 3 was 2.33 (SE = 0.06) and 1.82 (SE = 0.01), respectively. The decrease in CFU counts between these times was not nearly as great as that observed for wettable powder-treated foliage (Fig 1).

Both treatment and day affected persistence of *B. bassiana* on foliage as measured by laboratory assays of treated leaves (treatment: $F = 11.0$; df = 2, 47; $P < 0.0001$; day: $F = 45.5$; df = 2, 47; $P < 0.0001$). For each treatment, efficacy of spores on treated foliage declined over time, as estimated by linear regression. For wettable powder at the lower rate (Fig. 1a): Angular-transformed percentage mortality = 1.03 - 0.15[day] ($r^2 = 0.59$; SE = 0.09 and 0.03 for the 2 parameters, respectively; $F = 28.2$; df = 1, 18; $P < 0.0001$). For wettable powder at the higher rate (Fig. 2b): Angular-transformed percentage mortality = 1.21 - 0.14[day] ($r^2 = 0.59$; SE = 0.08 and 0.03 for the 2 parameters, respectively; $F = 28.0$; df = 1, 18; $P < 0.0001$). For emulsifiable suspension: Angular-transformed percentage mortality = 1.54 - 0.17[day] ($r^2 = 0.74$; SE = 0.11 and 0.03 for the 2 parameters, respectively; $F = 31.7$; df = 1, 10; $P < 0.0001$).

These trials indicate the potential for including *B. bassiana* in an integrated management program for *P. xylostella*. It is even more appropriate at this time to explore its use because populations of *P. xylostella* have developed resistance to many other available insecticides. We are not aware of any reports of resistance to this or any other fungus for any arthropod, and in previous studies we found no cross-resistance of *B. bassiana* to a formulated *B. thuringiensis* subspecies *kurstaki* product (Shelton et al. 1998). In that study we also demonstrated that *B. bassiana* could be 1 tool in an overall program of *P. xylostella* management because it could be used to provide transplants free of infestations of *P. xylostella*. Treating crucifers with *B. bassiana* at the seedling stage offers several

Table 4. Efficacy of *B. bassiana* strain GHA applied to cabbage infested with 3rd- to 4th-instar *P. xylostella*

Treatment	Rate ($\times 10^{13}$ spores/ha)	No. of applications	Live larvae[a]	$P <$[b]
Untreated	—	—	15.9 ± 3.8	1.0
WP carrier only	—	2	7.7 ± 2.3	0.1
ES carrier only	—	1	7.7 ± 2.5	0.1
Mycotrol WP	5.0	2	4.3 ± 1.2	0.01
Mycotrol WP	2.5	2	7.7 ± 2.1	0.1
Mycotrol WP	5.0	1	5.1 ± 1.9	0.02
Mycotrol WP	2.5	1	8.7 ± 2.2	0.2
Mycotrol ES	14.0	1	5.9 ± 1.6	0.03
Ambush 2EC	(0.11 kg [AI]/ha)	1	0.8 ± 0.2	0.001

[a] Average number of live larvae ± SEM per sample of 3 plants per plot. Larval counts were square-root transformed before ANOVA.
[b] Significance of difference from untreated control by the Dunnet test (Wilkinson et al. 1996).

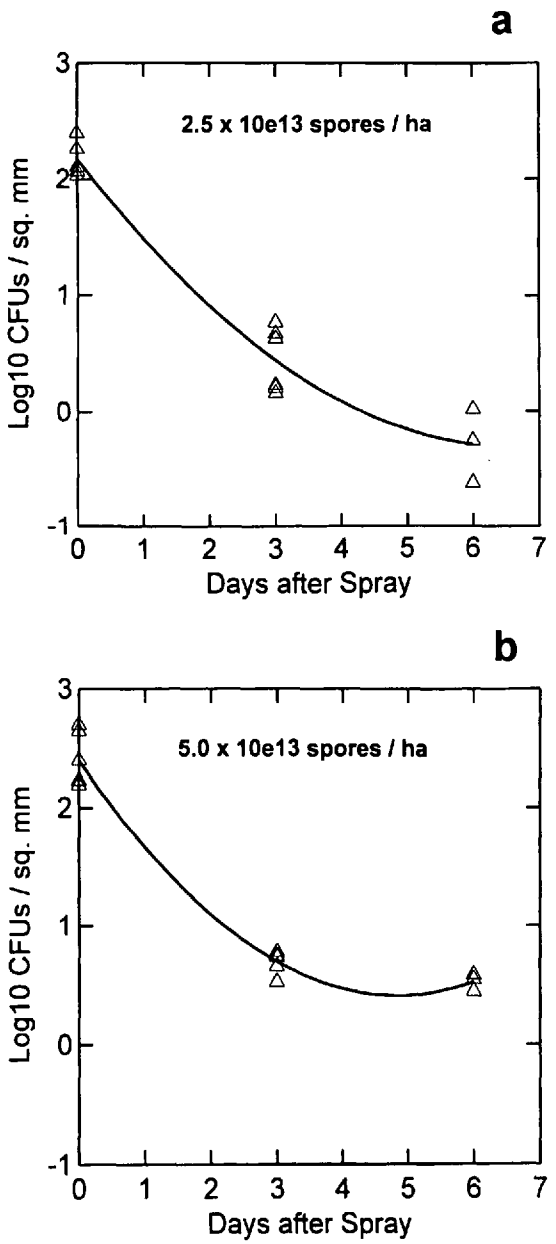

Fig. 1. Recovery of *B. bassiana* CFUs from cabbage leaf discs collected immediately after spraying and at 2 later times following a single application. (a) Plots treated with wettable powder at 2.5×10^{13} spores per hectare. (b) Plots treated with wettable powder at 5.0×10^{13} spores per hectare. See text for equations and statistics.

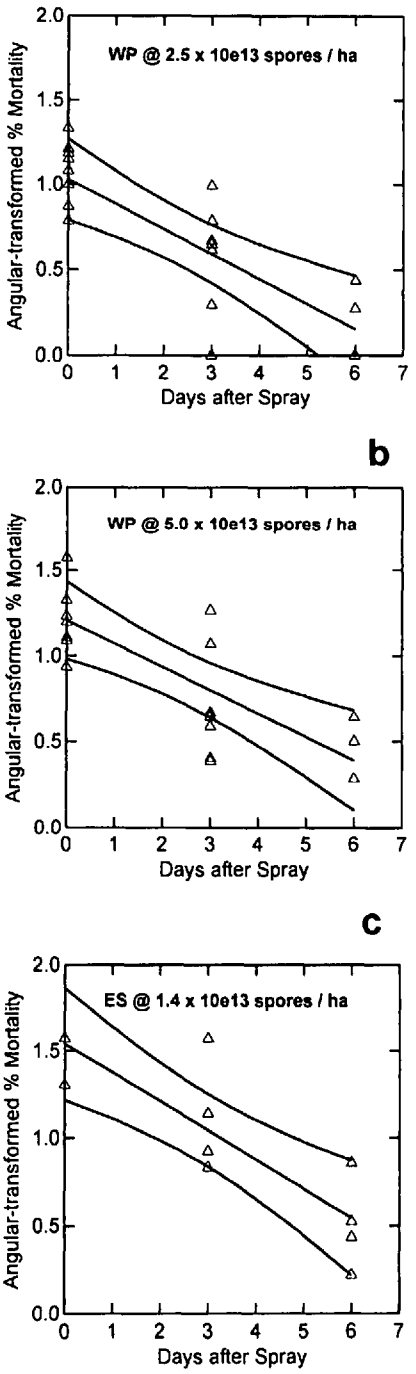

Fig. 2. Efficacy of *B. bassiana* spores on field-collected cabbage leaves against laboratory-reared *P. xylostella* larvae. Leaves collected immediately after spray with *B. bassiana* and at 2 later times following a single application. (a) Plots treated with wettable powder at 2.5×10^{13} spores per hectare. (b) Plots treated with wettable powder at 5×10^{13} spores per hectare. (c) Plots treated with emulsifiable suspension at 1.4×10^{14} spores per hectare. All mortality was due to *B. bassiana* mycosis. See text for equations and statistics.

advantages to seedling producers and crucifer growers (Shelton et al. 1998).

Our growth chamber experiment showed that a commercial formulation of *B. bassiana* was not greatly influenced by temperature or humidity within the range we tested. Our greenhouse tests demonstrated that either of 2 formulations were effective. Finally, our field trials indicated that commercial formulations of *B. bassiana* could provide significant control when

applied to 2nd, 3rd, and 4th instars infesting cabbage plants. The recommended action threshold for *P. xylostella* and other lepidopteran larvae in New York is at 30% infestation for crucifers with 1–28 true leaves (Cornell Cooperative Extension 1998). In our field trials applications of *B. bassiana* resulted in infestation rates below the action threshold when applied to younger larvae (approximately 1 larva per sample of 3 plants; Table 3) but not older larvae (Table 4). Additional trials are being done using improved formulations and modified application schemes, including the alternation of applications of chemical and microbial insecticides, to optimize control of *P. xylostella*.

While the emulsifiable suspension formulation at 14×10^{13} spores per hectare provided excellent control, the rate may be cost-prohibitive under field rather than greenhouse conditions. It should also be noted that the emulsifiable suspension formulation at this rate provided control similar to that obtained with the wettable powder formulations at 2.5 and 5×10^{13} spores per hectare applied once. If this product is to be registered for control of *P. xylostella* on cabbage, it would be appropriate to conduct additional trials to determine the optimum formulations and rates.

Persistence of the spores from the wettable powder formulation appears to be similar to many *B. thuringiensis* products which generally provide control for 3–4 d after application. In this study, persistence, as estimated by recovery of viable spores and efficacy of treated foliage for larvae, was rate-dependent. Application to foliage within the crop canopy may improve persistence because of the protection from germicidal UV rays (see Furlong and Pell 1997 for discussion). These findings must be considered when determining appropriate application methods and rates for registration of *B. bassiana* for control of *P. xylostella*.

Our studies on persistence also provide some insight into how the larvae contact the spores. In the growth chamber tests and the laboratory persistence assays, the leaves were treated and then larvae were added. Those larvae which died must have contacted the inoculum by crawling on the leaf surface. In addition, all 3 instars that were exposed on foliage were susceptible to *B. bassiana* infection (Tables 3 and 4; Vandenberg et al. 1998). These factors indicate that timing of application may not be so critical for control of *P. xylostella*. This may be important because later season populations of *P. xylostella* tend to have a mixture of instars.

From these studies and our related work with crucifer transplants (Shelton et al. 1998), it appears that *B. bassiana* may be a new tool for control of *P. xylostella*. Additional studies are needed to determine season-long control of *P. xylostella* as well as it effects on the other pests of crucifers and the natural enemies in the system. Our preliminary studies (unpublished data) indicate that this fungus can provide control of other lepidopteran pests of cabbage.

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