Efficacy and Persistence of *Beauveria bassiana* and Other Fungi for Control of Diamondback Moth (Lepidoptera: Plutellidae) on Cabbage Seedlings[1]

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Abstract The diamondback moth, *Plutella xylostella* (L.), is a key pest of crucifers worldwide. Resistance by diamondback moths to chemical insecticides and *Bacillus thuringiensis* Berliner toxins highlights the need for alternative controls. Crucifer growers often depend on seedlings produced in screenhouses and later transplanted to the field. Commercial seedlings can be contaminated with pesticide-resistant *P. xylostella,* leading to control problems in the field. We evaluated the efficacy of *Beauveria bassiana* (Balsamo) Vuillemin applied as conidia in Mycotrol® WP in three experiments by evaluating insect control, damage reduction, and fungus persistence on treated leaves of cabbage seedlings. In one experiment, we also evaluated *Metarhizium anisopliae* Metschnikoff (Sorokin) applied as conidia in Bio-blast® and *Paecilomyces fumosoroseus* (Wize) Brown & Smith applied as freshly-cultivated unformulated blastospores. We observed significant reductions, compared to controls, in insect populations and damage ratings when Mycotrol was applied once- or twice-weekly. Mycotrol treatments were as effective as a *B. thuringiensis* product in preventing damage when three spray nozzles were used to insure adequate pesticide coverage. Mycotrol persisted on treated leaves in screenhouses for more than 2 wks, and mycosis of larvae reared on these leaves was >50% 7 d after a single application of fungus. Treatment with either *M. anisopliae* or *P. fumosoroseus* resulted in no significant reduction in insect numbers or damage, but each fungus persisted on leaves and caused mycosis in lab-reared larvae more than 2 wks after treatment. Mycotrol can provide an option for control of *P. xylostella* larvae on seedlings, and may be especially useful in a resistance management program.

Key Words *Plutella xylostella,* insecticide resistance, transplants, cabbage, *Beauveria bassiana, Metarhizium anisopliae, Paecilomyces fumosoroseus*

The diamondback moth, *Plutella xylostella* (L.), is a key pest of crucifers worldwide; annual control costs exceed $1 billion (Talekar and Shelton 1993). *Plutella xylostella* has evolved resistance to synthetic organic insecticides, growth regulators and some toxins of *Bacillus thuringiensis* Berliner (Bt) in several important crucifer-growing areas of the world (Talekar and Shelton 1993). Biologically-based insecticides with alternative modes of action and which complement existing IPM programs are sorely needed. Recent work in Malaysia with *Beauveria bassiana* (Balsamo)

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Vuillemin and *Paecilomyces fumosoroseus* (Wize) Brown & Smith has shown promise for controlling *P. xylostella* in the field (Ibrahim and Low 1993). It is generally considered that *P. xylostella* is more problematic in hot, dry weather (Talekar and Shelton 1993), but dry conditions may be less than ideal for efficacy of an entomophagous fungus.

Crucifer production in many areas of the world relies on growing seedlings in a greenhouse or screenhouse and then transplanting them into the field. In a recent study, Shelton et al. (1996) noted the problem of seedlings being contaminated with *P. xylostella* larvae. In that study some sources of commercial transplants were contaminated with *P. xylostella* at levels of more than 30 larvae per 100 transplants with larvae being >200-fold resistant to methomyl, a commonly used insecticide. When contaminated plants with resistant insects are shipped to growers and transplanted to the field, serious control failures can result. Management of insects on small plants prior to transplantation presents opportunities to avoid season-long problems in the field (Shelton et al. 1996). Entomopathogenic fungi may be effective because transplants are generally grown under conditions of relatively high humidity with frequent watering—conditions considered to be more amenable for fungal infection of insects. Laboratory studies (Vandenberg and Ramos 1996, Vandenberg et al. 1998b) have noted the potential for control of *P. xylostella* by *B. bassiana*.

Mycotrol® WP (strain GHA, Mycotech Corp., Butte, MT) is a biopesticide with the fungus *B. bassiana* as the active ingredient. Two formulations of Mycotrol (WP, wettable powder, and ES, emulsifiable suspension) are currently registered for control of certain Orthoptera and Coleoptera on rangelands and Homoptera and thrips on a variety of crops. Because this product is currently produced and labelled on some crops, it may be possible to expand the label to include *P. xylostella* on transplants if the product is effective. Isolates of *Metarhizium anisopliae* Metschnikoff (Sorokin) have proven potential against the diamondback moth in preliminary screening trials (Vandenberg and Ramos 1997). Bio-Blast® (strain ESC-1, Ecoscience, East Brunswick, NJ) is a registered formulation of *M. anisopliae* for use against termites. Blastospores of *P. fumosoroseus,* produced in liquid medium, are efficacious against the silverleaf whitefly, *Bemisia argentifolia* (Gennadius) (Jackson et al. 1997), the Russian wheat aphid, *Diuraphis noxia* (Mordvilko) (Vandenberg et al. 1998a), and the diamondback moth (Cantone and Vandenberg 1995). The objective of this study was to examine the efficacy, timing and persistence of fungal biocontrol agents, compared with other alternatives, for control of *P. xylostella* larvae and larval damage on cabbage seedlings.

Materials and Methods

Plants. Broccoli (variety 'Green Goliath'; W. Atlee Burpee & Co., Warminster, PA) was seeded into 128-cell flats of Metro mix 350 (Griffin Greenhouse Supply, Auburn, NY) and maintained in a greenhouse at 25 ± 5° C with a 16:8 h (L:D) photoperiod. Flats were moved to screenhouses (5 m × 3 m × 2.5 m) when the first true leaves appeared. For the efficacy trials each flat represented one of four replications of each treatment. Additional flats were seeded for fungal persistence studies for each experiment as described below. The screenhouse had no heating or supplemental lighting, a condition similar to many transplant operations in southern states.

Insects. *Plutella xylostella* larvae were reared on diet according to Shelton et al. (1993) prior to release in the screenhouse. For Experiments 1 and 2, a population

established in Geneva, NY, in 1988 and susceptible to insecticides was used. For Experiment 3, the insects were derived from a field-collected Bt-resistant line (Perez et al. 1997). After the plants were in the screenhouse, approximately 200 adult *P. xylostella* were released and allowed to oviposit for 5 to 10 d. After 7 d the flats were removed from the screenhouse and treated outside. After treatment, flats were returned to the screenhouse and arranged randomly. Approximately 200 additional adults were released and further oviposition was allowed to occur for 7 d. Subsequent treatments also were applied outside.

At the end of the 2- or 4-wk experimental period, damage rating and insect counts were done. Plants were evaluated for injury by selecting five plants from five or six different sites within each flat and rating each plant for feeding injury. Percentage damage was scored at one of five levels: 0, 25, 50, 75 and 100%. Ratings were assessed in a manner similar to that used by Dapsis and Ferro (1982). For insect counts, the number of live larvae was recorded for each plant. Angular transformed percentage data and square root-transformed insect counts were subjected to analysis of variance procedures and when differences were significant, an LSD ($P < 0.05$) statistic was calculated. Three screenhouse experiments were done during the spring, summer and fall of 1996. Specific protocols for each of three experiments are described below.

Experiment 1. Treatments were initiated on 6 June and consisted of *B. bassiana* Mycotech strain GHA (Mycotrol WP) at 4.9×10^{13} spores per ha treated once weekly, *B. bassiana* at 4.9×10^{13} spores per ha treated twice weekly, permethrin (Ambush 2 EC, Zeneca, Wilmington, DE) at 0.11 kg AI per ha once weekly, *B. thuringiensis* subsp. *kurstaki* (Javelin WG, 6.4% AI, Sandoz, Des Plaines, IL) at 1.1 kg AI per ha once weekly, a water-treated control, and an untreated control. Treatments were applied with a CO_2-pressurized backpack sprayer with a single hollow cone nozzle (HC 12, R & D Sprayers, Inc, Opelousas, LA) directed downward from above the plant canopy and delivering 280.47 liters per ha at 2.81 kg cm^2 at 3.2 kph. Weekly treatments were applied on 6 and 13 June and biweekly treatments on 6, 10, 13 and 17 June. Damage evaluations and insect counts were done on 20 June.

Experiment 2. Treatments were initiated on 15 July and were identical to Experiment 1 except for the addition of a weekly treatment of Mycotrol base formulation (without AI). To increase the coverage of the sprays, treatments were applied to each flat through three hollow cone nozzles (same specifications as above): one nozzle was directed downward and one nozzle was directed from each side of the seedlings. Weekly treatments were applied on 15 and 22 July and the biweekly treatments on 15, 18, 22 and 25 July. Damage evaluations and insect counts were done on 29 July.

Experiment 3. Treatments were initiated on 21 October and application methods were identical to Experiment 2. Two additional treatments were included: *M. anisopliae* Ecoscience strain ESC-1 (Bio-blast) at 1.4×10^{12} conidia per ha applied weekly and *P. fumosoroseus* blastospores (Jackson et al. 1997) at 4.9×10^{13} spores per ha applied weekly. Bio-blast was applied at a lower rate because of limited product availability. The controls consisted of a Mycotrol base formulation (without AI) weekly and an untreated control. Weekly treatments were applied on 21 and 28 October and 4 and 11 November and the biweekly treatments on 21, 24 and 28 October and 4, 7, 11 and 14 November. Applications were extended over 4 wks due to cooler weather and consequent slower development of larvae (data not shown). Immediately after the application on 4 November, the flats were moved to a greenhouse at 25 ± 10° C

with a 16:8 h (L:D) photoperiod. Damage evaluations and insect counts were done on 20 November.

Temperature and relative humidity. Ambient conditions were recorded daily (minimum, maximum and average) at the site of the experiments. Average temperature and relative humidity were calculated for the period of each experiment.

Fungal persistence. For all fungus treatments and untreated controls, leaves from each of two seedlings per flat were removed at 0 (post-spray), 3 or 4, 7, 10 or 11 and 14 d after treatment and placed individually in 150-mm Petri dishes. Twelve second-instar (0.2 to 0.4 mg) *P. xylostella* larvae from the Geneva-1988 colony were added to each dish. Dishes were sealed with parafilm and incubated at 25 ± 1° C with a 15:9 h (L:D) photoperiod. After 7 d, mortality was assessed and dead larvae were removed and incubated in dishes of 1.5% water agar with moistened filter paper for 24 h to confirm fungal infection. In Experiment 3, to assess possible accumulation of viable spores of *B. bassiana,* leaf samples were collected 3 d after each of four consecutive twice-weekly treatments with Mycotrol in Experiment 3. Larvae were added to leaves and incubated as described above. Data were analyzed using analysis of covariance and regression analysis to determine effects of sample day, experiment and fungal species. Percentage mortality data were subjected to the angular transformation prior to analysis.

Results and Discussion

Damage assessment. We observed significant differences in damage ratings among treatments in all 3 experiments (Table 1; for Experiment 1: $F = 2.77$; df = 5, 18; $P < 0.0001$; for Experiment 2: $F = 2.57$; df = 6, 21; $P < 0.0001$; and for Experiment 3: $F = 2.57$; df = 6, 21; $P < 0.0001$). In Experiment 1, Ambush provided a significantly lower damage rating than any other treatment while Javelin was significantly better than either Mycotrol treatment. Mycotrol applied twice weekly was significantly better than when applied once weekly. Based on these results, we modified the spraying technique to provide better coverage and in Experiment 2 the performance of Mycotrol was improved accordingly. While the performance of Ambush and Javelin stayed relatively the same between the first and second trials, the performance of Mycotrol when applied twice weekly improved approximately 48% and was no longer significantly different from Javelin. Mycotrol applied once weekly still resulted in significantly greater damage than the twice-weekly treatment. In Experiment 3 both Mycotrol treatments provided significantly better control than Javelin against the Bt-resistant insects used in this experiment. In Experiment 3 we observed no difference between the weekly and twice-weekly treatments, but this may have been due to the additional treatments applied over the longer time course. The performance of Mycotrol applied twice weekly in Experiments 2 and 3 was nearly identical, and better than in Experiment 1. This may have been due, in part, to the use of three spray nozzles (and not one) in last two experiments. In Experiment 3 neither *M. anisopliae* or *P. fumosoroseus* resulted in significant reduction in damage compared to controls.

Insect counts. We observed significant differences in insect counts among treatments in all three experiments (Table 1; for Experiment 1: $F = 2.77$; df = 5, 18; $P < 0.0001$; for Experiment 2: $F = 2.57$; df = 6, 21; $P < 0.0001$; and for Experiment 3: $F = 2.57$; df = 6, 21; $P < 0.0001$). Treatment with Ambush resulted in the lowest insect counts in Experiment 1, and Mycotrol applied once weekly was not significantly different from the untreated control. In Experiment 2 all insecticide treatments and the

Table 1. Control of *Plutella xylostella* larvae and their damage on cabbage seedlings.

Experiment*	Treatment**	Mean Damage Rating†	Mean Larvae per Plant†
1	Ambush 2EC (0.11 kg AI/ha)	20.0 a	0.00 a
	Javelin WG (0.07 kg AI/ha)	32.4 b	0.32 b
	Mycotrol WP (4.9×10^{13} spores/ha—twice weekly)	58.7 c	0.32 b
	Mycotrol WP (4.9×10^{13} spores/ha)	86.4 d	0.70 c
	Water	99.6 e	0.37 b
	Untreated	98.8 e	0.62bc
2	Ambush 2EC (0.11 kg AI/ha)	23.7 a	0.00 a
	Javelin WG (0.07 kg AI/ha)	27.7ab	0.01 a
	Mycotrol WP (4.9×10^{13} spores/ha—twice weekly)	30.7 b	0.01 a
	Mycotrol WP (4.9×10^{13} spores/ha)	41.0 c	0.02 a
	Mycotrol WP Carrier Only	59.5 d	0.11 a
	Water	82.5 e	0.28 b
	Untreated	98.5 f	0.52 c
3	Javelin WG (0.07 kg AI/ha)	59.0 b	1.51 b
	Mycotrol WP (4.9×10^{13} spores/ha—twice weekly)	29.8 a	0.31 a
	Mycotrol WP (4.9×10^{13} spores/ha)	33.8 a	0.34 a
	Bio-blast (1.4×10^{12} spores/ha)	59.2 b	1.30 b
	Paecilomyces fumosoroseus (4.9×10^{13} spores/ha)	63.8 b	1.65 b
	Bio-blast Carrier Only	63.8 b	1.69 b
	Untreated	60.4 b	1.42 b

*Single spray nozzle used in Experiment 1; three nozzles used in Experiments 2 and 3 (see text for description of spray boom). Insects from Bt-susceptible strain used in Experiments 1 and 2; insects from a Bt-resistant strain were used for Experiment 3 (see text for origins). All treatments were applied once weekly for 2-4 weeks except as indicated for twice-weekly applications of Mycotrol.

**Mycotrol AI is spores of *Beauveria bassiana;* Bio-blast AI is spores of *Metarhizium anisopliae.*

†Means for each Experiment followed by the same letter are not significantly different by analysis of variance (LSD test, $P < 0.05$).

Mycotrol WP carrier-only treatment resulted in significantly reduced insect counts compared to controls, although the lowest numbers were recorded for the four insecticide treatments. In Experiment 3, only the Mycotrol treatments, both once and twice weekly, resulted in significantly reduced insect counts.

Fungal persistence. There was a significant decline in persistence of *B. bassiana* over time, judged by insect bioassay ($F = 181.14$; df = 1, 54; $P < 0.0001$), but there was no significant difference among experiments ($F = 0.90$; df = 2, 54; $P < 0.41$). Analysis of covariance showed no significant interaction between experiment and sample day ($F = 0.72$; df = 2, 54; $P < 0.49$), indicating no difference among the slopes for the regression of angular-transformed mortality on sample day. Data were then combined for analysis and are shown in Fig. 1. The combined regression equation was: [Angular-transformed mortality = 1.58 – 0.10 (Sample day)], where $F = 152.42$; df = 1, 58; $P < 0.0001$; SEM for the intercept = 0.07; SEM for the slope = 0.01, and $r^2 = 0.72$. In all 3 experiments leaf samples taken immediately after spray elicited 100% mortality among laboratory-reared larvae and >75% mortality for leaf samples collected 3 d after treatment (Fig. 1). No fungal mortality was observed among control larvae.

For Experiment 3, considering all three fungal species, there was a significant effect of sample day ($F = 121.34$; df = 1, 90; $P < 0.0001$) and a significant difference among the three fungal species ($F = 4.41$; df = 2, 90; $P < 0.015$). There was a

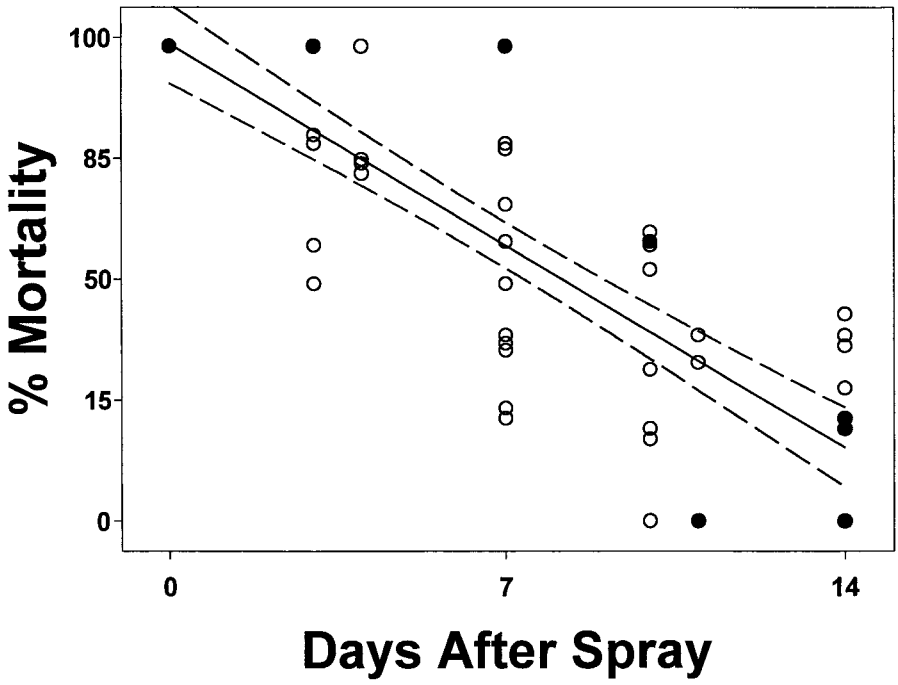

Fig. 1. Mortality of laboratory-reared diamondback moth larvae on excised leaves following a single application of Mycotrol (AI is *Beauveria bassiana*) on cabbage seedlings in a screenhouse. Solid circles represent multiple data points. Combined data from three experiments. Analysis done on angular-transformed percentage mortality data. Regression line shown with 95% confidence interval. See text for equation and statistics.

marginally significant difference among the slopes, judged by analysis of covariance ($F = 2.73$; df = 2, 90; $P < 0.07$), so the regressions are shown separately in Fig. 2. For *B. bassiana,* the regression was: [Angular-transformed mortality = 1.32 – 0.08 (Sample day)], where $F = 96.96$; df = 1, 30; $P < 0.0001$; SEM for the intercept = 0.09; SEM for the slope = 0.007, and $r^2 = 0.80$. For *M. anisopliae,* the regression was: [Angular-transformed mortality = 0.88 – 0.04 (Sample day)], where $F = 23.76$; df = 1, 30; $P < 0.0001$; SEM for the intercept = 0.12; SEM for the slope = 0.008, and $r^2 = 0.04$. For *P. fumosoroseus,* the regression was: [Angular-transformed mortality = 0.87 – 0.05 (Sample day)], where $F = 27.34$; df = 1, 30; $P < 0.0001$; SEM for the intercept = 0.12; SEM for the slope = 0.008, and $r^2 = 0.51$. Bio-blast and *P. fumosoroseus* provided averages of 54% and 80% mortality, respectively, to larvae exposed to leaves immediately after the spray, but declined more rapidly and did not persist as long as Mycotrol (Fig. 2). Laboratory trials have shown *M. anisopliae* to be highly efficacious against *P. xylostella* (Vandenberg and Ramos 1997), and further field trials at higher rates are warranted. In the case of *P. fumosoroseus* the lack of persistence may be due to the relative fragility of the blastospore stage and the absence of any formulation additives to the spore suspension. Further tests with formulated blastospore preparations are needed.

There was no significant effect of the number of applications (up to four) of Mycotrol in Experiment 3 on larval mortality from leaf samples taken 3 d after each treatment ($F = 1.40$; df = 3, 44; $P < 0.26$). The average (SEM) percent mortality for each successive sample was 55 (8), 42 (10), 31 (9), and 35 (8).

The average temperatures over the course of Experiments 1 to 3, respectively, were 20° C (SD = 2), 20° C (SD = 2) and 5° C (SD = 4), and the average relative humidities were 86% (SD = 7), 84% (SD = 5), and 84% (SD = 9). Higher values for damage ratings and larval counts in Experiment 3 (Table 1) may be due, in part, to the lower average temperature. In a related laboratory study (Vandenberg et al. 1998b) we found highest infection rates of *P. xylostella* larvae by *B. bassiana* between 20 and 30° C, with lower infection observed at both higher and lower temperatures. For field applications, optimum performance of *B. bassiana* strain GHA will probably be observed in this temperature range. Although relative humidities as low as 34% were recorded, relative humidity exceeded 95% for a portion of every day. This period of high humidity probably provides sufficient moisture for the germination and infection process to commence.

Higher UV values are related to decreased conidial survival of fungal pathogens under field conditions (Carruthers et al. 1988, Daoust and Pereira 1986, Inglis et al. 1993). Although Mycotrol has no UV protectants, it has demonstrated field persistence with conidial survival and infectivity for 7 d on the undersides of melon and cabbage leaves (S. Jaronski, pers. commun.; Vandenberg et al. 1998c). Extended conidial survival in screenhouse conditions found in these experiments, with minimal exposure to UV radiation because of a solid roof overhead, will probably facilitate control under commercial conditions.

These results provide promise for the use of Mycotrol for protection of crucifer transplants which may be attacked by *P. xylostella.* In these trials insect pressure was intense and most likely well above that experienced in commercial situations. Despite this, in our experiments Mycotrol applied twice weekly provided control equal to a common Bt product when test insects were susceptible to all pesticides. Mycotrol applied once weekly gave significantly better results than control treatments. Against Bt-resistant insects in Experiment 3, Mycotrol applied once or twice weekly gave

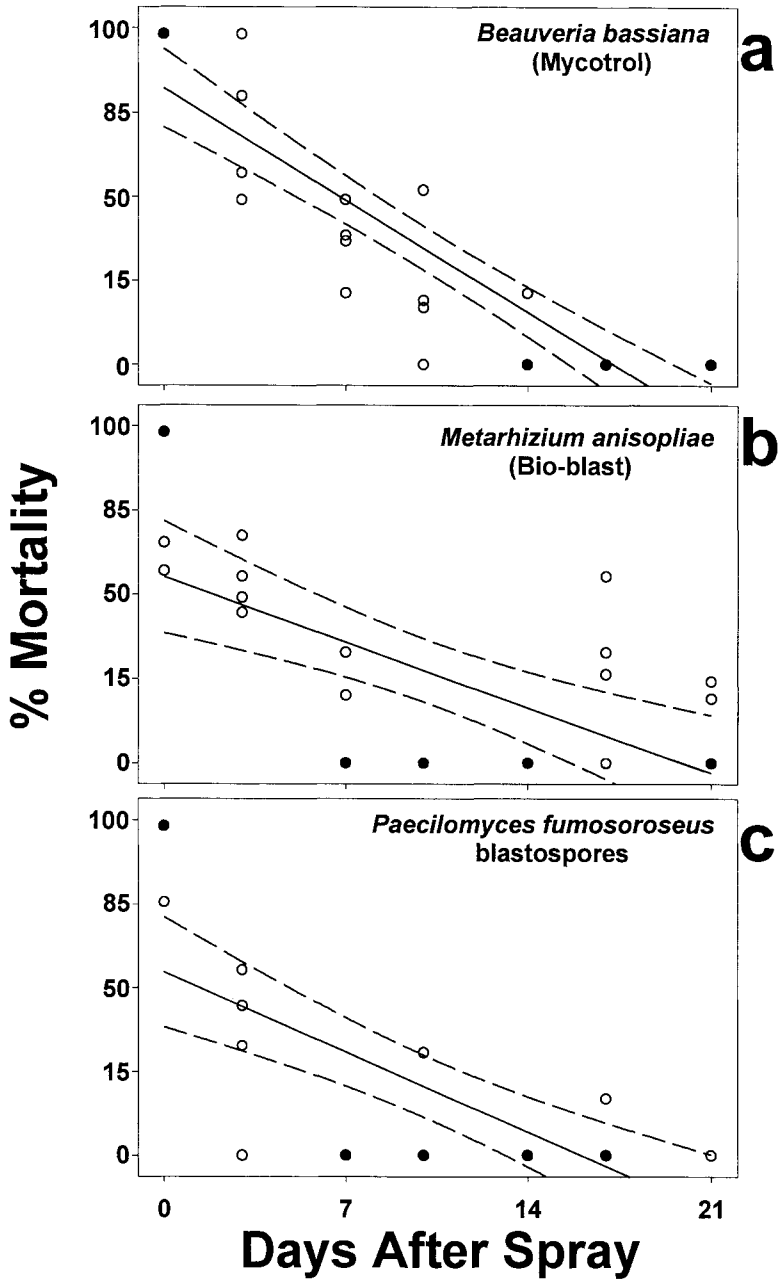

Fig. 2. Mortality of laboratory-reared diamondback moth larvae on excised leaves following a single application of (a) Mycotrol (AI is *Beauveria bassiana*), (b) Bio-Blast (AI is *Metarhizium anisopliae*), or (c) *Paecilomyces fumosoroseus* in Experiment 3 on cabbage seedlings in a screenhouse. Solid circles represent multiple data points. Analysis done on angular-transformed percentage mortality data. Regression lines shown with 95% confidence intervals. See text for equations and statistics.

superior protection (Table 1). In several areas of the southeastern United States, *P. xylostella* has become resistant to some pyrethroid, organophosphate and carbamate insecticides as well as Bt (Shelton et al. 1993b); a product such as Mycotrol may provide the only control.

The use of transplants is increasing among commercial crucifer growers due to increasing seed costs, the value of individual plants, and benefits of crop uniformity made possible by transplants. Production of transplants includes many factors including the cost of insecticides. The cost of Mycotrol may be relatively high ($15 per treatment per acre for field use at the time of this study) in comparison to many standard insecticides, but insecticides represent a relatively small proportion of transplant production costs. Furthermore, because transplants are concentrated in relatively small areas, the amount of insecticide applied per unit can be reduced versus field applications. *Plutella xylostella* larvae appear more susceptible to *B. bassiana* in later instars, perhaps due to shedding of fungal spores with molted cuticle (Vandenberg et al. 1998b). This may prove an advantage for Mycotrol over Bt products which require higher doses to achieve control of older larvae (Perez et al. 1997). These factors, coupled with the increased risk of insect resistance to the other available products, may facilitate pesticide label expansion for Mycotrol and use of *B. bassiana* to control *P. xylostella* on commercially-produced crucifer transplants.

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