BIOLOGICAL CONTROL 5, 581–590 (1995)

Influence of Ultraviolet Light Protectants on Persistence of the Entomopathogenic Fungus, *Beauveria bassiana*

G. DOUGLAS INGLIS,*,†,[1] MARK S. GOETTEL,* AND DAN L. JOHNSON*

*Agriculture and Agri-Food Canada Research Centre, P.O. Box 3000, Lethbridge, Alberta, Canada T1J 4B1; and †Centre for Pest Management, Simon Fraser University, Burnaby, British Columbia, V5A 1S6

Received August 22, 1994; accepted January 19, 1995

The effect of ultraviolet light (uv) protectants on persistence of conidia of the entomopathogenic fungus *Beauveria bassiana* was investigated in laboratory and field environments. The survival of conidia applied in water onto glass coverslips or crested wheatgrass (*Agropyron cristatum*) leaves was reduced by greater than 95% after 15 min exposure to uv-B radiation in a controlled environment. Substitution of oil for water increased the survival of conidia on both substrates. However, conidial survival in oil was more pronounced on glass (74% mortality after 60 min) than on leaves (97% mortality after 60 min). The decreased protection provided by oil on leaves was attributed to spreading and/or absorption of the oil by the leaf tissues. None of 21 potential sunscreen formulations were toxic to nongerminated conidia *in vitro*. On wheatgrass leaves, 5 of the 12 water-compatible and two of the nine oil-compatible formulations enhanced survival of conidia after 3 h exposure to uv-B radiation in a controlled environment. Four water-compatible and three oil-compatible sunscreen adjuvants were subsequently tested in a repeated field experiment. The water-compatible fluorescent brightener, Tinopal LPW (conidial survival slopes of −2.1 and −1.7 in trials one and two, respectively), and a clay emulsion (slopes of −2.5 and −2.0) significantly increased survival of conidia compared to the water control (slopes of −3.3 and −2.7), whereas Congo Red (slopes of −3.1 and −2.8) and the optical brightener, Blankophor BSU (slopes of −4.2 and −3.7), were ineffective. Conidial survival in the field was not enhanced by the three oil-compatible adjuvants tested (oxybenzone, octyl-salicylate, and ethyl-cinnamate). The use of uv-B protectants in formulations can increase conidial survival and may enhance the efficacy of *B. bassiana* for controlling insect pests in epigeal habitats.

KEY WORDS: *Beauveria bassiana;* persistence; ultraviolet radiation; uv-B; sunscreens; protectants; crested wheatgrass; *Agropyron cristatum;* formulation oil.

INTRODUCTION

The entomopathogenic fungus *Beauveria bassiana* (Bals.) Vuill. has shown considerable potential for the management of insect pests (Feng *et al.*, 1994). Grasshoppers are economically important pests in arid agroecosystems, and recently the pathogenicity of *B. bassiana* has been demonstrated in a field environment against grasshoppers (Johnson and Goettel, 1993). However, *B. bassiana* conidia are hyaline and rapidly killed by sunlight (Daoust and Pereira, 1986; Inglis *et al.*, 1993). Since a threshold of inoculum is required to cause beauveriosis in insects, the inactivation of conidia by sunlight could seriously decrease the efficacy of conidia applied on foliage. If epigeal habitats are to be targeted for the management of insect populations with *B. bassiana,* methods to increase the persistence of conidia are needed.

The ultraviolet radiation-B (uv-B; 280-320 nm) component of sunlight is detrimental to all microorganisms (Tevini, 1993). A number of substances have been used to protect and enhance the persistence of entomopathogenic viruses (Ignoffo and Batzer, 1971; Shapiro *et al.*, 1983; Martignoni and Iwai, 1985; Shapiro, 1989, 1992), *Bacillus thuringiensis* Berliner (Morris, 1983; Cohen *et al.*, 1991), the entomopathogenic fungus *Metarhizium flavoviride* Gams & Rozsypal (Moore *et al.*, 1993), and the nematode *Steinernema carpocapsae* (Weiser) (Nickle and Shapiro, 1992) exposed to artificial uv-B radiation. However, the efficacy of uv-B protectants for increasing the persistence of fungal propagules in a field environment has not been previously studied. Therefore, the objectives of this study were to: (1) test and compare water and paraffinic oil formulations for uv protection of *B. bassiana* conidia; (2) screen a number of potential uv protectants in the laboratory; and (3) test their efficacy in a field environment.

[1] To whom correspondence should be addressed at Agriculture and Agri-Food Canada, Research Centre, P.O. Box 3000, Lethbridge, Alberta, Canada T1J 4B1. Fax: (403) 382-3156.

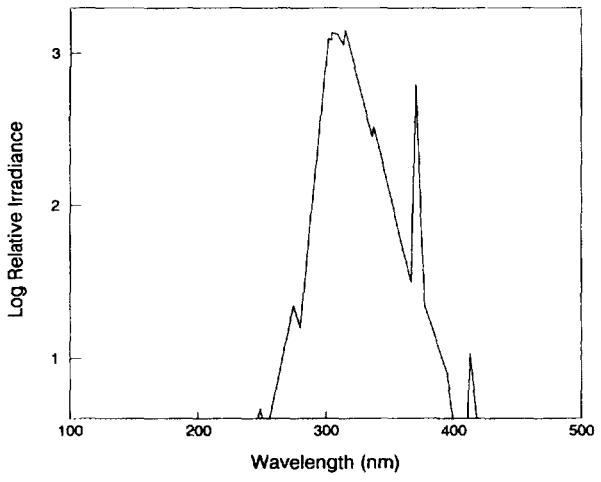

FIG. 1. Spectral distribution of irradiative energy from the Ultra-Lum uv-B fluorescent bulb used for irradiating *Beauveria bassiana* conidia.

MATERIALS AND METHODS

Conidial Inoculum

Immediately before use, dry conidia of *B. bassiana* (GHA strain, supplied by Mycotech Corp., Butte, MT) were suspended in sterile deionized water or in paraffinic formulation oil using a Kontes mechanical pestle or a Potter–Elvehjem homogenizer. In the laboratory experiments, a paraffinic emulsifiable oil was used, whereas a paraffinic oil flowable (no emulsifier added) was used in the field experiment. Conidial concentrations were estimated with a hemocytometer and adjusted as required.

Influence of Substrate

The survival of conidia exposed to uv-B radiation on leaves and on glass was compared. Conidia (2×10^5 conidia/μl) suspended in water or in oil were pipetted (1 μl) onto sterile round coverslips (13 mm in diameter) and onto the surface of leaf pieces (approximately 2×0.5 cm) of field-collected crested wheatgrass (*Agropyron cristatum* L.) attached to a white plastic tray using double-sided tape. The coverslips and leaf pieces were then placed 10 cm below a uv-B fluorescent bulb (Ultra-Lum, Carson, CA) for 0, 15, 30, 45, or 60 min at 25 ± 1°C. Radiation from this bulb ranges in wavelength from 260 to 400 nm, with a peak near 300 to 320 nm (Fig. 1). Intensity of uv-B radiation was measured using a UVX radiometer (UVP Inc., San Gabriel, CA) equipped with a UVX-31 sensor (310 nm peak); uv flux at a distance of 10 cm ranged from 601 to 675 μW/cm^2 along the length of the bulb. Following exposure, leaf pieces and coverslips were individually placed in 5 ml of 0.01 *M* phosphate buffer with 0.05% Tween 80 (pH, 7.0) in 20-ml scintillation vials and washed at ambient temperature for 2 h on a rotary shaker at 300 rpm. The wash solution was then diluted in a 10-fold dilution series and 100-μl aliquots for each dilution were spread on a semiselective oatmeal–dodine agar medium (consisting of 17.5 g oatmeal agar, 2.5 g agar, 0.45 g Cyprex (dodine), 2.5 mg crystal violet, 0.2 g penicillin, and 0.5 g streptomycin in 500 ml of deionized water) (Chase *et al.*, 1986). Cultures were incubated at 25°C for 6 to 7 days and the number of colony-forming units (CFU) were enumerated at the dilution yielding 30 to 300 CFU per dish. Conidial survival relative to the control (time$_0$) was calculated as (CFU T_0 − CFU T_x/CFU T_0) × 100. An initial experiment indicated that incubation of conidia in water and oil at 25°C in the dark for up to 6 h had no effect on conidial viability.

Sunscreens

The adjuvants tested were divided into water- or oil-compatible compounds. Water-compatible adjuvants consisted of eight stilbene brighteners provided by Dr. M. Shapiro, USDA-ARS, Beltsville, Maryland; Congo Red (Sigma Chemical Co., St. Louis, MO); and diethanolamine-4-methoxycinnamate (Nipasorb D; Graessorb D; Nipa Laboratories, Clwyd, UK). In addition, an attapulgite clay formulation (consisting of 15% oil (v/v), 12% clay (w/v) and 73% deionized water) and a 5% oil–water emulsion formulation (v/v) were tested. The oil-compatible adjuvants used were: butyl-methoxy-dibenzoylmethane (Parsol 1789; Givaudan & Co. Ltd., Surrey, UK); ethyl trans-cinnamate (Sigma); 4-isopropyl-dibenzoylmethane (Eusolex 8020; Merck, Darmstadt, Germany); magnesium silicate (Florisil 60-100 mesh; Fisher Scientific, Edmonton, AB, Canada); octyl-*p*-methoxycinnamate (Parsol MCX; Givaudan); octyl-salicylate (Graessorb S; Nipa Laboratories); 2-hydroxy-4-methoxybenzophenone (oxybenzone; Sigma); and 2,2-dihydroxy-4-methoxybenzophenone and 2,2-hydroxy-4-octoxybenzophenone supplied by Mycotech.

Solubility and Toxicity of Sunscreens

The solubilities of the sunscreens were determined in water and oil at room temperature (20 to 22°C). The maximum sunscreen concentration tested was 5%; saturated solutions at room temperature were used if sunscreens were insoluble at 5%. To measure possible toxicity, nongerminated conidia (2×10^5 conidia/μl) were suspended in each of the sunscreens. After 12 h at 25°C, 1-μl aliquots were pipetted onto sterile coverslips, and the coverslips were washed and CFU enumerated on oatmeal–dodine agar as previously described.

Sunscreen Selection

Conidia were suspended in each of the formulations so that the final concentration was 2×10^5 conidia/

μl; sunscreen concentrations were the same as for the toxicity experiment. Within 15 min, conidial suspensions (1 μl) were pipetted onto the surfaces of leaf pieces taped to a plastic tray and the leaf pieces were exposed to uv-B radiation for 1.5 and 3.0 h. The leaf pieces were washed and viable conidia were enumerated on oatmeal–dodine agar as previously described. Control treatments consisted of conidia applied in oil or water onto leaf pieces that were either exposed to uv-B radiation or maintained in the dark for the same period.

Field Evaluations

Conidia in various uv protectants were sprayed on crested wheatgrass in a field at the Agriculture and Agri-Food Canada Research Centre, Lethbridge. The field had been seeded in 1989 at a rate of 11.1 kg/ha with rows 17.5 cm apart and mowed in April 1992, 3 months prior to commencement of the experiment. Treatments were arranged in a randomized complete block design with three replicate plots measuring 3.0 by 1.5 m. The target concentration was 3.0×10^{13} conidia/ha or 1.4×10^{10} conidia/plot. The water treatments were Tinopal LPW (Calcofluor white; M_2R; 5% w/v), Blankophor BSU (5% w/v), Congo Red (5% w/v), clay (12% w/v), and water alone. Tinopal LPW was obtained by adjusting the pH of Blankophor, BBH, to 9.5 with 1 *N* potassium hydroxide. The oil treatments consisted of oxybenzone (5% w/v), ethyl-cinnamate (5% v/v), octyl-salicylate (5% v/v) and oil alone. An uninoculated control treatment was also included.

Water-compatible formulations were applied at a rate of 100 litres/ha (45 ml/plot), using a compressed CO_2 (40 PSI) bicycle sprayer (R&D Sprayers Inc., Opelousas, LA) equipped with three 015-F80 nozzles (Lurmark Ltd., Longstanton, Cambridge, UK). To obtain the optimal spray pattern, the height of the boom was adjusted according to the height of the wheatgrass canopy. Oil-compatible formulations were applied at a rate of 5 litres/ha (2.25 ml/plot) with an ultralow volume (ULV) spinning disk sprayer (Micron Sprayers Ltd., Bromyard, UK) operated at 7000 rpm. To eliminate drift, plots were enclosed in a polyethylene tent ($3.0 \times 1.5 \times 1.5$ m) during the ULV spray application. Water- and oil-sensitive papers were randomly placed on the soil surface in selected plots to evaluate the spray distribution. Conidia were applied at times of low wind velocity (<4 m/s) on the morning of July 28, 1993 for trial one and August 12 for trial two.

Ten leaves from the top of the canopy were randomly collected from the centre of each of the three replicate plots. Times of sampling were immediately after (time 0), and 1, 2, 4, 6, 8, 12, and 16 days postapplication. Care was taken to choose older leaves that would have been present at the time of conidial application for the 6- to 16-day sample times. Within 1 h of collection, leaves were transported to the laboratory in plastic bags and each of the 10 leaves per replicate were aseptically cut across the laminae into pieces of about 1 cm long. Propagules were recovered from the leaf pieces using the wash, dilution-spread-plate technique on oatmeal–dodine agar as described previously. Following the washing the total area of the leaf pieces were determined with a leaf area meter (Model 3100, Li-Cor Inc., Lincoln, NE) and the mean number of CFU/cm^2 of leaf area was calculated. Sample leaf areas per plot averaged 6.07 and 6.72 cm^2 in trials one and two, respectively. To confirm the identity of *B. bassiana,* representative colonies were isolated, grown in slide culture, and examined microscopically.

Weather Data

Mean hourly incoming solar radiation (300 to 2800 nm), temperature, relative humidity, precipitation, and wind direction and velocity were recorded at a weather station adjacent to the field plots. The pyranometer malfunctioned 4 and 5 days after application of conidia in trial one. Hours of bright sunshine during these two days were 13.4 and 8.7 h, respectively (Environment Canada, Lethbridge). Using hours of bright sunshine, theoretical total incoming solar radiation at ground level (Q_s theoretical) on these days was calculated using the equations of Baier and Robertson (1965) and Robertson (1968) as 3.17×10^4 kJ/m^2 for Day 4 and 2.40×10^4 kJ/m^2 for Day 5. These values were higher than those recorded by the pyranometer (Q_s actual) for days with comparable hours of bright sunshine. Therefore, Q_s theoretical was plotted against Q_s actual for the period of July 28 to August 28, 1993. The coefficient of determination (r^2) observed was 0.96 and the equation used to describe this relationship was Q_s actual = 3967.9 + 1.08(Q_s theoretical); standard errors of the mean (SE) were 903.4 and 0.044 kJ/m^2 for a and b, respectively. From the equation, Q_s was estimated as 2.58×10^4 kJ/m^2 for Day 4 and 1.87×10^4 kJ/m^2 for Day 5.

Statistical Analyses

All computations were performed using the GLM, REG, and TTEST procedures (SAS Institute, 1988). Residuals were plotted against predicted values and where necessary the appropriate transformations were used to normalize the data. The conidial population data were always $\log_{10}$ transformed, and $\log_{10}$-values for the means and SE are presented throughout the text. In all instances, SE were calculated from individual treatments and are presented in parentheses. In two cases in the substrate selection experiment, the percentage reduction data (Table 1) were arcsine-transformed, but untransformed means and SE are presented in Table 1.

All experiments in controlled environments were arranged as completely randomized designs. The substrate selection experiment was analyzed as a split plot in time with two levels of formulation and substrate and five levels of time. This experiment was conducted three times for the water formulation and twice for the oil formulation; data from the trials were combined for analysis. The toxicity and selection experiments were repeated once, and with the exception of the clay formulation treatment, they were analyzed using one-way ANOVA. In conjunction with a significant F test, Tukey's studentized range test ($\alpha = 0.05$) was used to separate means. For the clay formulation, comparisons were made using the TTEST procedure.

The field experiment was arranged as a randomized complete block design. Water- and oil-compatible formulations were analyzed separately as a split plot in time. When a significant ($P \leq 0.05$) interaction was observed for formulation and time, pairwise comparisons of the slopes of conidial persistence between the control and test formulation were conducted using analysis of covariance with $\log_{10}$-transformed time used as the covariate. For the SAS REG procedure, the mean persistence data for each formulation were used to fit linear models; $\log_{10}$-transformation of the x-axis was used for water-compatible formulations in both trials and for the oil-compatible formulations in trial one but not in trial two. In addition to time, the predictability of cumulative solar radiation on conidial survival was examined by regression analysis. In most instances, no *B. bassiana* conidia were recovered from leaf segments collected from untreated plots, and when conidia were recovered, it was at very low levels (<10 CFU/cm^2). Therefore, the uninoculated control treatment was excluded from the analyses of conidial persistence.

RESULTS

Influence of Substrate

On both leaves and coverslips, droplets of water were localized and evaporated within 15 min of placement. Oil droplets (4 to 5 mm in diameter) covered a larger area than did water droplets (1.5 to 2.0 mm in diameter) on coverslips. On wheatgrass leaves, oil spread rapidly across the lamina and an oil sheen was usually observed.

When exposed to uv-B radiation in the laboratory, significant interactions were observed between formulation (oil and water) and duration of exposure ($F = 8.2$; $df = 4,112$; $P \leq 0.0001$), and formulation and substrate ($F = 13.0$; $df = 1,19$; $P = 0.0019$). Comparisons between the oil and water formulations for individual substrates indicated that conidial survival was enhanced in oil on glass (Table 1); formulation ($F = 65.4$; $df = 1,5$; $P = 0.0005$), time ($F = 23.4$; $df = 4,56$; $P \leq 0.0001$), and the interaction between formulation and time ($F = 8.5$; $df = 4,56$; $P \leq 0.0001$) were significant. Although there was no interaction ($F = 1.95$; $df = 4,56$; $P = 0.11$) between formulation and time, more conidia ($F = 8.3$; $df = 1,5$; $P = 0.035$) were recovered from leaves treated with conidia in oil (averaged over time) than with conidia in water.

Comparisons between substrates for individual formulations indicated that the survival of conidia applied in oil on coverslips was greater ($F = 21.5$; $df = 1,5$; $P = 0.0056$) than the survival of conidia applied in oil to leaves (Table 1). Time alone was highly significant ($F = 8.6$; $df = 4,40$; $P \leq 0.0001$), and there was no interaction ($F = 1.3$; $df = 4,40$; $P = 0.29$) between time and substrate. In water, there was no difference ($F = 0.74$; $df = 1,9$; $P = 0.41$) in survival of conidia applied to either substrate (Table 1). As with oil, there was no interaction ($F = 2.0$; $df = 4,72$; $P = 0.10$) between time and substrate and time alone was highly significant ($F = 46.6$; $df = 4,72$; $P \leq 0.0001$).

Solubility and Toxicity of Sunscreens

Of the adjuvants tested, 5 of 10 and 6 of 9 were highly soluble in water and oil (>5% w/v), respectively. The Blankophor brighteners, BBH, DML, HRS, LPG, and RKH were marginally soluble in water, and Florisil, HOB, and HMB exhibited low solubility in oil. Saturated formulations (room temperature) of the marginally soluble adjuvants were subsequently tested for uv protection. None of the water- ($F = 0.85$; $df = 10,11$; $P = 0.60$) or oil-compatible ($F = 1.19$; $df = 7,8$; $P = 0.40$) adjuvants tested were toxic to nongerminated conidia of *B. bassiana* after 12 h incubation at 25°C. The increased pH of Blankophor, BBH (9.5), required to enhance its solubility in water, had no effect on conidial viability.

Sunscreen Selection

After 1.5 h exposure to uv-B radiation, conidial survival in 9 of 11 water-compatible formulations was greater ($\alpha = 0.05$) than that of conidia exposed to uv-B radiation in water alone (Table 2). Conidial survival was equal to that of nonexposed conidia applied in water in all but three of the formulations. Five of the adjuvants protected conidia ($\alpha = 0.05$), exposed to uv-B radiation for 3 h. These included Congo Red and the optical brighteners, BSU, BBH, P167, and Tinopal LPW; BBH was tested at a concentration of only 0.25% (w/v). The clay formulation treatment was analyzed separately. There was no difference in survival ($T = 1.51$; $df = 10$; $P = 0.16$) between conidia exposed to uv-B for 1.5 h (3.74, SE = 0.20 log CFU/leaf) and conidia maintained in the dark (4.14, SE = 0.17 log CFU/leaf). In contrast, conidial survival in clay was reduced ($T = 2.57$; $df = 10$; $P = 0.028$) after 3.0 h exposure to uv-B.

TABLE 1

Influence of Formulation and Substrate on Survival of *Beauveria bassiana* Conidia Exposed to uv-B Radiation

Duration of exposure (min)	Glass (SE)		Leaves (SE)	
	$\log_{10}$ cfu[a]	% Reduction[b]	$\log_{10}$ CFU[a]	% Reduction[b]
Water formulation				
0	3.96 (0.06) a[c]	—	4.11 (0.06) a	—
15	2.46 (0.11) b	96.0 (0.78) a[d]	2.36 (0.28) b	96.1 (1.0) a[d]
30	1.59 (0.27) bc	99.0 (0.27) b	1.70 (0.30) bc	98.8 (0.42) ab
45	0.66 (0.34) c	99.3 (0.41) b	1.66 (0.31) bc	98.6 (0.66) b
60	1.12 (0.31) c	99.4 (0.26) b	0.74 (0.30) c	99.7 (0.10) b
Oil formulation				
0	3.99 (0.08) a	—	3.75 (0.15) a	—
15	3.92 (0.07) ab	22.4 (6.0) a	3.24 (0.22) a	49.2 (18.0) a
30	3.58 (0.20) ab	49.9 (16.4) ab	2.64 (0.54) ab	75.7 (7.1) ab
45	3.32 (0.27) ab	62.2 (14.5) ab	2.80 (0.24) ab	82.2 (5.2) ab
60	3.13 (0.26) b	74.4 (9.3) b	1.79 (0.38) b	97.4 (0.89) b

[a] Conidia ($\log_{10}$ colony-forming units (CFU)) recovered from glass coverslips or crested wheatgrass leaves. Values in parentheses following means represent standard errors of the means.

[b] Percentage reduction was calculated as $((CFU_{T0} - CFU_{Tx})/CFU_{T0})100$.

[c] Means not followed by the same letter within each formulation-substrate group are significantly different ($\alpha = 0.05$) according to Tukey's studentized range test. The experiment was conducted three times for the water formulation ($n = 10$) and two times for the oil formulation ($n = 6$).

[d] Data were arcsine transformed.

From leaves treated with conidia in clay and exposed to uv-B for 3.0 h, 4.19 (0.11) log CFU/leaf were recovered compared to 4.55 (0.089) log CFU/leaf from nonexposed leaves. Congo Red, clay, Tinopal LPW, and Blankophor, BSU, were selected for evaluation in the field experiment.

None of the nine oil-compatible formulations tested enhanced ($\alpha = 0.05$) survival relative to oil alone after 1.5 h exposure to uv-B radiation (Table 3). After 3.0 h exposure to uv-B radiation, conidial survival in Parsol MCX and 2,2-hydroxy-4-octoxybenzophenone was superior ($\alpha = 0.05$) to the survival of conidia applied in

TABLE 2

Influence of Water-Compatible Formulation Adjuvants on Survival of *Beauveria bassiana* Conidia Applied to Leaves and Exposed to uv-B Radiation for 1.5 and 3.0 h

Formulation	Concentration (%)	$\log_{10}$ CFU/leaf[a] (SE)	
		1.5 h	3.0 h
BSU-Optical brightener (OB)[b]	5	4.68 (0.04) a[c]	4.41 (0.07) a[c]
Congo Red[b]	5	4.58 (0.03) a	4.45 (0.10) a
Tinopal LPW-OB[b]	5	4.46 (0.06) a	4.12 (0.12) ab
BBH-OB	0.25	4.35 (0.15) a	4.22 (0.12) ab
P167-OB	5	4.27 (0.31) a	4.43 (0.04) a
HRS-OB	2	4.13 (0.10) ab	3.49 (0.15) abc
LPG-OB	0.25	4.01 (0.14) ab	3.55 (0.23) abc
DML-OB	0.25	4.10 (0.05) ab	3.18 (0.32) bc
RKH-OB	0.25	2.86 (0.34) b	3.27 (0.32) abc
Diethanolamine-4-methoxycinnamate	5	1.16 (0.52) c	0.41 (0.41) d
Oil emulsion	5	0.74 (0.49) c	0.53 (0.53) d
Water	—	1.10 (0.52) c	2.77 (0.16) c
Water (no uv Exposure)	—	4.68 (0.06) a	4.44 (0.06) a

[a] Conidia ($\log_{10}$ CFU/leaf) recovered from crested wheatgrass leaves.

[b] Adjuvants selected for subsequent field evaluations.

[c] Means not followed by the same letter are significantly different ($\alpha = 0.05$), according to Tukey's studentized range test. The experiment was conducted two times ($n = 6$).

TABLE 3

Influence of Oil-Compatible Formulation Adjuvants on Survival of *Beauveria bassiana* Conidia Applied to Leaves and Exposed to uv-B Radiation for 1.5 and 3.0 h

Formulation	Concentration (%)	$\log_{10}$ CFU/leaf[a] (SE) 1.5 h	3.0 h
Oxybenzone[b]	5	3.71 (0.17) ab[c]	1.40 (0.47) bcd[c]
Ethyl-cinnamate[b]	5	3.44 (0.17) ab	1.19 (0.38) bcd
2,2-Dihydroxy-4-methoxybenzophenone	2	3.36 (0.22) ab	1.75 (0.35) abc
Parsol MCX	5	3.21 (0.23) ab	2.49 (0.50) ab
Octyl-salicylate[b]	5	3.16 (0.41) ab	0.85 (0.35) bcd
Eusolex	5	2.96 (0.35) ab	1.64 (0.37) abcd
Parsol 1789	5	2.82 (0.04) ab	0.00 (0.00) d
2,2-Hydroxy-4-octoxybenzophenone	4	2.31 (0.77) b	2.37 (0.13) ab
Florisil	2	2.17 (0.48) b	1.19 (0.38) bcd
Oil	—	2.51 (0.17) b	0.50 (0.50) cd
Oil (No uv exposure)	—	4.41 (0.18) a	3.36 (0.14) a

[a] Conidia ($\log_{10}$ CFU/leaf) recovered from crested wheatgrass leaves.
[b] Adjuvants selected for subsequent field evaluations.
[c] Means not followed by the same letter are significantly different ($\alpha = 0.05$), according to Tukey's studentized range test. The experiment was conducted two times ($n = 6$).

oil alone. On the basis of availability and a previous report of their efficacy in protecting *Metarhizium flavoviride* conidia from artificial uv-B radiation (Moore *et al.*, 1993), oxybenzone, ethyl-cinnamate, and octyl-salicylate were selected for evaluation in the field experiment.

Field Evaluations

Conditions of incoming solar radiation, temperature, precipitation, and relative humidity fluctuated within and between trials (Fig. 2). Total incoming solar radiation was 3.72×10^5 kJ/m^2 in trial one and 25.1% less (2.79×10^5 kJ/m^2) in trial two. Hourly incoming solar radiation, averaged over the 16 days of the trials (daylight hours), was 1430 (74.9) and 1327 (62.6) kJ/m^2, respectively. Mean hourly temperatures were 16.2 (0.29) and 15.2 (0.23) °C, and relative humidities averaged 67.8 (1.1) and 75.8 (0.94) % in trials one and two, respectively. Five periods of light precipitation (<3.0 mm per event) were recorded in trial one. In trial two, 51 mm of rain fell 3 to 5 days after application, followed by two additional periods of light rain (<1 mm).

Of the five water-compatible formulations, there was no difference ($F = 1.37$ and 1.60; $df = 4,8$; $P = 0.33$ and $P = 0.26$) in conidial populations among the formulations immediately after application (T_0) in either trial one or two; populations ranged from 4.10 (0.12) to 5.05 (0.21) log CFU/cm^2. For all the water-compatible formulations, conidial survival declined logarithmically over time (Figs. 3 and 4), and the persistence data were fitted to linear regressions following log-transformation of both the CFU and the time data. Coefficients of

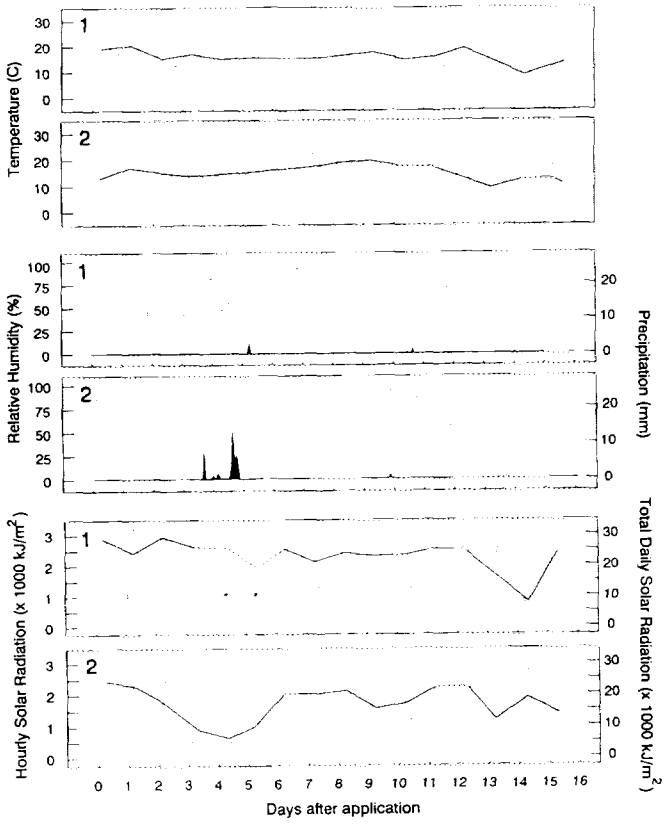

FIG. 2. Hourly (dotted lines) and mean (solid lines) temperature (°C), relative humidity (%), precipitation (peaks), hourly (dotted lines) and total daily (solid lines) solar radiation (kJ/m^2; 300–2800 nm) during trials one and two. Asterisks represent missing solar radiation data; daily solar radiation during this period (dashed line) was estimated using hours of bright sunshine.

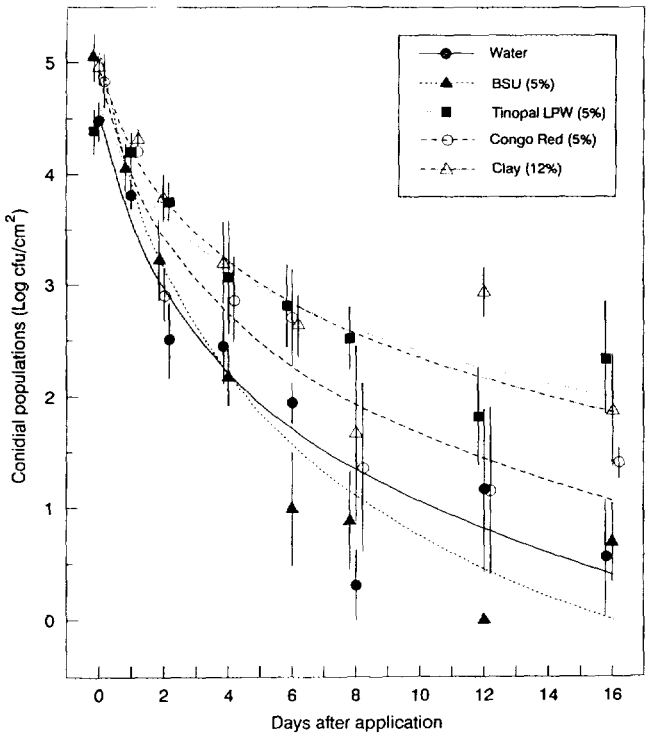

FIG. 3. Persistence of *Beauveria bassiana* conidia in water-compatible sunscreens on crested wheatgrass in trial one (July 28 to August 13, 1993). Populations were quantified as $\log_{10}$ colony-forming units (CFU)/cm^2 of leaf area and vertical lines represent standard errors of means (n = 3). To avoid superimposition of standard error bars, means are offset along the x-axis.

determination (r^2) ranged from 0.84 to 0.95 and from 0.85 to 0.98 for trials one and two, respectively (Table 4). Slopes of conidial persistence for the formulations ranged from −1.7 to −4.2, and both slopes (T = −5.6 to −16.9; df = 6; $P \leq 0.0013$) and y-intercepts (T = 12.0 to 36.5; df = 6; $P \leq 0.0001$) were significantly different from zero. For individual treatments, neither slopes nor y-intercepts differed (F = 0.24 to 2.96; df = 1,44; $P \geq 0.09$) between trials. Although a strong relationship was observed between cumulative solar radiation and conidial persistence, light was generally a less effective predictor of conidial survival (r^2 = 0.67 to 0.98) than was time (log-transformed). However, in trial two for the clay formulation, a stronger relationship was observed between conidial persistence and light (r^2 = 0.98) than with time (r^2 = 0.85).

In both trials, time (F = 67.3 and F = 75.8; df = 7,70; $P \leq 0.0001$) and formulation (F = 9.97 and F = 20.6; df = 4,8; P = 0.0034 and P = 0.0003) were significant, as were the interactions (F = 1.84 and F = 1.91; df = 28,70; P = 0.021 and P = 0.013) between them. Pairwise comparisons of the formulations with the control treatment using analysis of covariance indicated that Tinopal LPW (F = 6.19 and F = 8.42; df = 1,44; P = 0.0167 and P = 0.0058) and clay (F = 4.30 and F = 3.97; df = 1,44; P = 0.0439 and P = 0.0527) enhanced survival of conidia in both trials (Figs. 3 and 4) and that neither formulation was superior (F = 0.60 and F = 0.62; df = 1,44; P = 0.44 and P = 0.43). Congo Red did not affect (F = 0.21 and F = 0.04; df = 1,44; P = 0.65 and P = 0.85) the persistence of conidia in either trial. Although similar (F = 2.88; df = 1,44; P = 0.097) to the control treatment in trial one, the survival of conidia in BSU was less (F = 7.74; df = 1,44; P = 0.0079) than in water in trial two.

Immediately after application, conidial populations were similar (F = 0.10 and F = 0.29; df = 3,6; P = 0.96 and P = 0.82) in the four oil-compatible formulations in both trials. In contrast to the water-compatible formulations, slopes of conidial persistence differed between the trials. In trial one, best fit regression analysis required a $\log_{10}$-transformation of both CFU and time data, and slopes of conidial persistence ranged from −3.1 to −3.8 (Table 4). In contrast, only the CFU and not the time data required log-transformation in trial two; slopes ranged from −0.20 to −0.23. In both trials, all slopes (T = −6.4 to −24.8; df = 6; $P \leq 0.0003$) and y-intercepts (T = 14.7 to 62.1; df = 6; $P \leq 0.0001$)

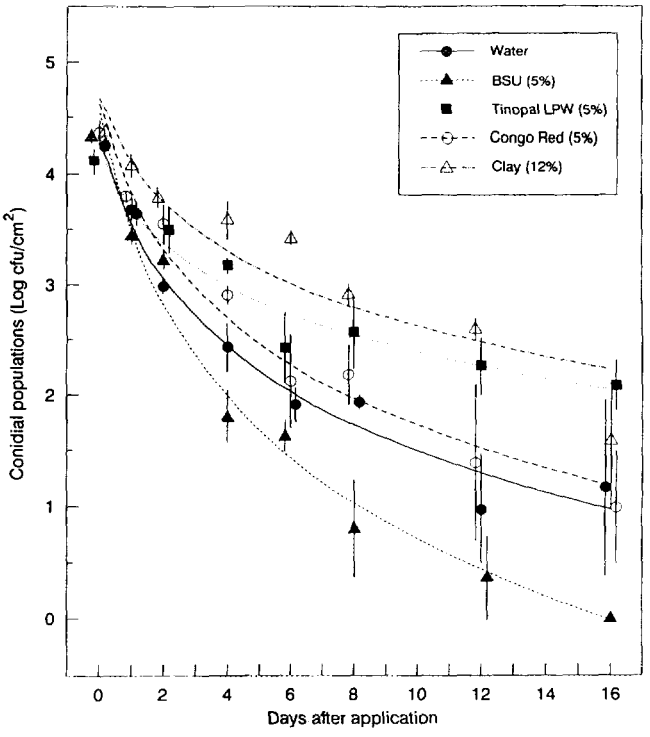

FIG. 4. Persistence of *Beauveria bassiana* conidia in water-compatible sunscreens on crested wheatgrass in trial two (August 12 to 28). Populations were quantified as $\log_{10}$ colony-forming units (CFU)/cm^2 of leaf area and vertical lines represent standard errors of means (n = 3). To avoid superimposition of standard error bars, means are offset along the x-axis.

TABLE 4

Linear Regression Data for *Beauveria bassiana* Conidial Persistence on Crested Wheatgrass Leaves in the Field Experiment[a]

	Trial one					Trial two				
	a	SE(a)	b	SE(b)	r^2	a	SE(a)	b	SE(b)	r^2
Water-compatible										
Water	4.50[b]	0.37	−3.33[b]	0.46	0.90	4.31	0.14	−2.71	0.17	0.98
BSU	5.08	0.32	−4.19	0.40	0.95	4.53	0.18	−3.68	0.22	0.98
Tinopal LPW	4.59	0.19	−2.11	0.24	0.93	4.18	0.12	−1.73	0.14	0.96
Congo Red	4.85	0.30	−3.08	0.37	0.92	4.62	0.16	−2.78	0.20	0.97
Clay	4.92	0.36	−2.49	0.44	0.84	4.66	0.27	−1.96	0.33	0.86
Oil-compatible										
Oil	5.03	0.31	−3.83	0.39	0.94	4.18	0.16	−0.20	0.02	0.94
Oxybenzone	5.08	0.21	−3.07	0.26	0.96	4.49	0.12	−0.23	0.02	0.97
Ethyl-cinnamate	5.06	0.18	−3.43	0.22	0.98	4.54	0.07	−0.23	0.01	0.99
Octyl-salicylate	4.89	0.33	−3.06	0.41	0.90	4.41	0.09	−0.22	0.01	0.98

[a] For the water-compatible formulations in both trials and the oil-compatible formulations in trial one, the x-axis was $\log_{10}$-transformed and the regression equation is: $\log_{10}$ CFU/cm^2 = $a + b$ ($\log_{10}$ days + 1), where $n = 8$. For the oil-compatible formulations in trial two, the x-axis was untransformed and the regression equation used is: $\log_{10}$ CFU/cm^2 = $a + b$ (days).

[b] All y-intercepts and slopes are significantly different from zero ($P \leq 0.01$).

were significantly different from zero. Coefficients of determination for the oil-compatible formulations ranged from 0.90 to 0.99 (Table 4). As with the water-formulations, a strong relationship was observed between cumulative light and conidial persistence for oil (r^2 = 0.85 and 0.94), oxybenzone (r^2 = 0.95 and 0.97), ethyl-cinnamate (r^2 = 0.95 and 0.99), and octyl-salicylate (r^2 = 0.74 and 0.98). For the oil and octyl-salicylate formulations in trial one, and all formulations in trial two, light was almost as good a predictor of conidial survival as was time.

For the oil-compatible formulations, time (F = 51.0 and F = 87.8; df = 7,56; $P \leq 0.0001$) but not formulation (F = 3.65 and F = 0.80; df = 3,6; P = 0.083 and P = 0.53) influenced conidial persistence, and there was no interaction (F = 0.59 and F = 0.44; df = 21,56; P = 0.91 and P = 0.98) between formulation and time (Figs. 5 and 6). Comparison of water and oil controls over time indicated no difference in conidial persistence in trial one (F = 0.48; df = 7,28; P = 0.84) or two (F = 1.1; df = 7,28; P = 0.36).

DISCUSSION

The impact of uv-B radiation on fungal populations in natural habitats has not been extensively studied. *B. bassiana,* a hyaline fungus, is soil-borne and is rarely isolated from plant foliage. Furthermore, the survival of *B. bassiana* conidia applied on foliage is very poor (Inglis *et al.,* 1993), and the most important parameter limiting survival of conidia in epigeal habitats appears to be sunlight (Daoust and Pereira, 1986; Inglis *et al.,* 1993). Ultraviolet radiation causes primary (i.e., nucleic acid mutations) and/or secondary (i.e., photoreactions) damage to exposed microorganisms, either of which may lead to cellular death (Tevini, 1993). We observed that conidia of *B. bassiana* are highly sensitive to artificial uv-B radiation but the mechanism causing death is unknown. It seems likely that the extreme sensitivity of *B. bassiana* to the uv-B portion of the solar spectrum limits its persistence in epigeal habitats. Formulation of *B. bassiana* to provide protection from ultraviolet light will be necessary to increase its survival and efficacy in epigeal habitats.

We found that Congo Red, clay, and all of the stilbene brighteners tested provided a degree of protection from artificial uv-B radiation (Table 2). Four of the adjuvants were tested in a field environment but only clay and the stilbene brightener, Tinopal LPW, consistently increased the persistence of *B. bassiana* conidia on wheatgrass leaves. Congo Red and the stilbene brightener, BSU, were ineffective. Stilbene brighteners readily absorb uv-B radiation and have previously been shown to protect nuclear polyhedrosis virus (NPV) occlusion bodies (Martignoni and Iwai, 1985; Shapiro, 1992), *Steinernema carpocapsae* (Nickle and Shapiro, 1992), and *B. thuringiensis* (Morris, 1983) from uv-B inactivation. Tinopal LPW has also been shown to enhance the virulence of NPV (Shapiro and Dougherty, 1993). In contrast to the uv-B absorbing adjuvants, clay acts as a sunlight blocker. Sunlight blockers have provided effective protection of viruses from uv-B radiation (Ignoffo and Batzer, 1971; Jaques, 1971; Shapiro *et al.,* 1983), and the use of blockers such as clay, starch, or carbon may be preferable because they are either environmentally innocuous or easily decomposed.

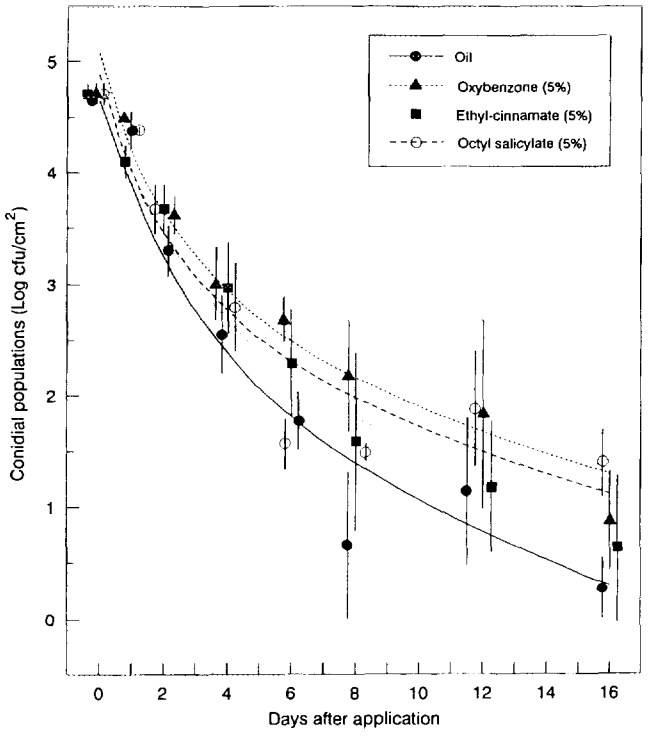

FIG. 5. Persistence of *Beauveria bassiana* conidia in oil-compatible sunscreens on crested wheatgrass in trial one (July 28 to August 13, 1993). Populations were quantified as $\log_{10}$ colony-forming units (CFU)/cm^2 of leaf area and vertical lines represent standard errors of means (n = 3). To avoid superimposition of standard error bars, means are offset along the x-axis.

Several entomopathogens, including *B. bassiana,* have been applied in oil at ultralow volumes in attempts to increase their efficacy (Feng *et al.,* 1994). Similarly to a previous field study (Inglis *et al.,* 1993), we found no marked increase in the survival of *B. bassiana* conidia applied in oil at ULV relative to conidia sprayed in water at conventional volumes. However, in our laboratory experiment, we observed that *B. bassiana* conidia exposed to uv-B radiation survived better in oil than in water on glass. Conidia of *M. flavoviride* in oil on glass were also found to be less sensitive to uv radiation (below 305 nm) than were conidia in water; this was attributed to absorption of uv radiation by the oil (Moore *et al.,* 1993). Although we observed that survival of conidia was also greater on leaves in oil than in water, conidial persistence in oil was substantially reduced on leaves relative to that on glass. Despite the presence of an oil sheen, we attributed the decreased efficacy of oil on leaves, at least in part, to the absorption of oil into mesophyll cells. Under field conditions, the volume of oil deposited on leaves at ULV is considerably less than the volume of oil (1 μl) that we pipetted onto the leaf segments in the laboratory. Therefore, it would be expected that the rate and degree of absorption of oil into leaf tissues applied at ULV would be greater, and the rapid absorption of oil into leaf tissues may explain the poor protection of *B. bassiana* conidia from uv-B radiation that we observed on leaves in the field experiment.

Oil-soluble sunscreens, which absorb uv-B radiation, have been developed for use in the cosmetic industry (Shaath, 1990). Several of these (oxybenzone, ethyl-cinnamate, and octyl-salicylate) were found to protect *M. flavoviride* conidia on glass (Moore *et al.,* 1993). Although several of the oil-compatible adjuvants we tested protected *B. bassiana* conidia from artificial uv-B radiation, they did not protect conidia under field conditions. Reasons for the differential efficacy of these adjuvants between the field and the laboratory are unknown. However, absorption of the oil carrier into the leaf tissues may have contributed to the decreased protection provided by these adjuvants in the field.

This study demonstrated that *B. bassiana* conidia are extremely sensitive to uv-B radiation and that the survival of conidia can be prolonged in field environments by using uv-B protectants. The results also confirm that solar radiation, and in particular the uv-B portion of the solar spectrum, is important in limiting

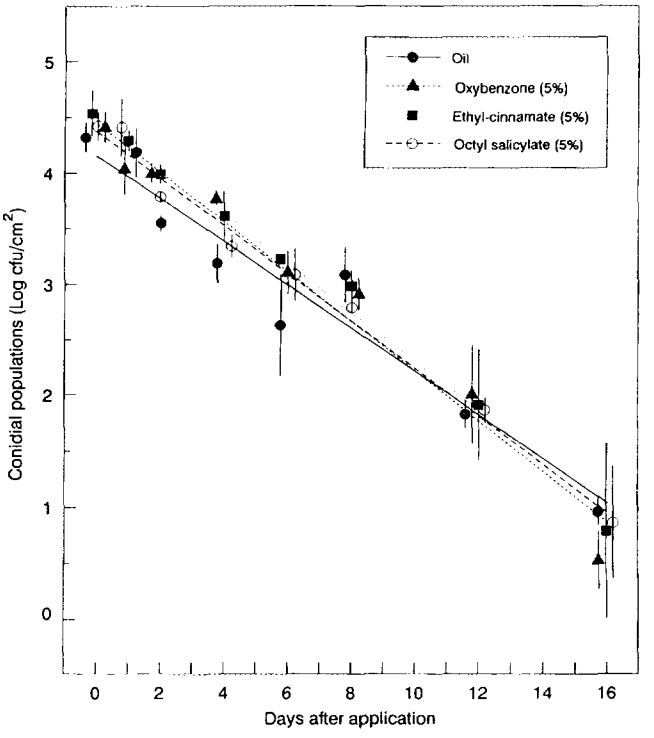

FIG. 6. Persistence of *Beauveria bassiana* conidia in oil-compatible sunscreens on crested wheatgrass phylloplanes in trial two (August 12 to 28). Populations were quantified as $\log_{10}$ colony-forming units (CFU)/cm^2 of leaf area and vertical lines represent standard errors of means (n = 3). To avoid superimposition of standard error bars, means are offset along the x-axis.

conidial survival in epigeal habitats. However, the utilization of uv-B protectants to increase the efficacy of insect control will depend on whether sunscreens prolong the survival of conidia sufficiently to enhance efficacy. Future research should focus on efficacy tests against insects and on the identification of more effective uv-B protectants and/or formulation strategies.

ACKNOWLEDGMENTS

We thank the following: (1) R. Feniuk, Agriculture and Agri-Food Canada (AAFC), Lethbridge, for assisting with the application of conidia, collection of leaves, isolation, and enumeration of CFU; (2) G. Duke, T. Danyk, R. Ouedraogo, AAFC, and Bonifacio Magalhaes, EMBRAPA/CENARGEN, Brasilia, Brazil, for assisting with the application of conidia; (3) T. Entz, AAFC for statistical advice; (4) Mycotech Corporation, Butte, Montana, for providing conidia, formulation oils, and several sunscreens; (5) Dr. M. Shapiro, USDA, Beltsville, Maryland; Nipa Laboratories, Sandycroft, Deeside, Clwyd, UK; Dr. Dave Moore, The International Institute for Biological Control, Ascot, Berks, UK, for supplying additional sunscreens; and (6) Drs. M. Shapiro, USDA-ARS, Beltsville, Maryland; C. Quimby, USDA-ARS, Boseman, Montana; H. D., Burges, UK; and J. R. Byers, AAFC, for critically reviewing the manuscript. The research was funded in part by a grant from Farming for the Future, Alberta Agricultural Research Institute, and a Pest Management Alternatives Office graduate student scholarship to G.D.I. This manuscript is Lethbridge Research Centre contribution No. 3879419.

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