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Effects of Temperature and Sunlight on Mycosis (*Beauveria bassiana*) (Hyphomycetes: Sympodulosporae) of Grasshoppers Under Field Conditions

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ABSTRACT The influence of environmental conditions on mycosis of grasshoppers caused by *Beauveria bassiana* (Balsamo) Vuillemin, was investigated. Despite the deposition of considerable quantities of conidia onto grasshoppers (6.7×10^3 colony-forming units [CFU] per nymph), *B. bassiana* did not significantly reduce field populations nor did it affect specific grasshopper taxa. Conditions were warm and sunny during the trial, and slopes of conidial persistence were equally poor on both grasshoppers and grass leaves. Small numbers of conidia (<2 CFU per grasshopper) were recovered from surface-sterilized grasshoppers 5–15 d after application indicating that if infection occurred, *B. bassiana* did not proliferate in the hemocoel. Considerable mycosis was observed in grasshoppers placed in cages in the greenhouse, but not in grasshoppers confined in cages adjacent to the field plots. Furthermore, the prevalence of disease in the greenhouse cages decreased with the sampling date but the onset of mycosis always occurred 3–4 d after collection, suggesting that environmental conditions in the greenhouse were responsible for the increased susceptibility of grasshoppers. Higher prevalence and more rapid development of disease were observed in grasshoppers placed in shaded cages (83–89%) than in cages exposed to full sunlight (0–15%) or protected from UVB radiation (1–43%); conidial survival was equally enhanced in the shaded and UVB-protected environments. Our results indicate that the poor efficacy of *B. bassiana* against rangeland grasshoppers was a result of conditions of temperature and light exposure (reduced grasshopper thermoregulation), and not the result of inadequate host targeting or pathogen virulence.

KEY WORDS *Beauveria bassiana*, grasshoppers, light, temperature, thermoregulation

GRASSHOPPERS ARE MAJOR pests of crops in arid agroecosystems, and existing control strategies rely almost exclusively on the use of chemical insecticides. However, recognition of the deleterious effects of pesticides have prompted the development of alternative, less obtrusive management strategies. The entomopathogenic fungus *Beauveria bassiana* (Balsamo) Vuillemin has shown considerable potential for the management of insects (Feng et al. 1994), and it is currently under investigation as a microbial control agent of acridids (Goettel et al. 1995). Infection is initiated by asexual spores (conidia) deposited onto the external integument of grasshoppers. In some field trials the application of *B. bassiana* conidia has resulted in substantial decreases in grasshopper populations (Johnson and Goettel 1993). In others, results have been less promising (Johnson et al. 1992, Lobo Lima et al. 1992, Inglis et al. 1996a). If *B. bassiana* is going to be used to effectively manage acridids, a better understanding of factors that contribute to the epizootiology of disease is necessary.

Recent evidence has suggested that environmental conditions limit the efficacy of *B. bassiana* in the field (Inglis et al. 1996a). Conidia are killed rapidly by exposure to sunlight, and in particular the UVB portion of the solar spectrum (Inglis et al. 1995a). In addition, acridids elevate their body temperatures higher than ambient by habitat selection, orientation to solar radiation, or both (Chappell and Whitman 1990, Heinrich 1993), and thermoregulation by grasshoppers reduced mycosis caused by *B. bassiana* in a controlled setting (Inglis et al. 1996b). The objective of this study was to identify the environmental parameters that limit the efficacy of *B. bassiana* against grasshoppers in field settings. To accomplish this, field trials were conducted to test the efficacy of *B. bassiana* against field populations of acridids, and to determine the influence of temperature and sunlight on mycosis. To study temperature and sunlight effects, we monitored disease in field-collected grasshoppers placed in greenhouse and exposed field cages at various times after condial application. We also monitored disease in field-collected grasshoppers placed in field cages that were shaded, protected from UVB radiation, or exposed to full spectrum sunlight; a parallel study was conducted with laboratory-inoculated grasshoppers to further evaluate the last goal.

Materials and Methods

Field Efficacy. The field site was located 28 km north of Coaldale, AB, Canada, on native short-grass prairie rangeland. Twelve plots were established and each plot was 3 ha (173 by 173 m), separated from each other by a minimum of 100 m. Treatments consisted of *B. bassiana* conidia, a carrier control and an untreated control arranged as a randomized complete block design with 4 blocks; the untreated control treatment was included to determine if the carrier alone affected grasshoppers. Dry conidia (strain GHA; Mycotech, Butte, MT) were suspended in a 1.5% (wt:vol) oil emulsion amended with 4% clay (wt:vol) immediately before application. Conidia (2.5×10^{13} conidia per hectare) and the carrier alone were applied at a rate of 112 liters/ha (Inglis et al. 1996a) on the morning of 12 July 1995.

Weather data were recorded at the Agriculture and Agri-Food Canada (AAFC) centers located at Lethbridge and Vauxhall; the Lethbridge and Vauxhall centers are located 49 km southwest and 33 km southeast from the field site, respectively. Mean hourly solar radiation (300–2,800 nm), temperature, and relative humidity were recorded at both sites and UVB radiation (280–320 nm) was only measured at Lethbridge. Daily precipitation and mean hourly wind speeds (height of 65 cm) at the field site were also recorded.

Conidial Deposition. Four sterile round glass coverslips (13 mm diameter) were spaced evenly on the bottom of a petri dish (9 mm diameter) with double-sided tape. Within each of the *B. bassiana* and carrier control plots, four, 4-m^2 subplots were established 70 m diagonally from the corners of the plot boundary. Dishes were placed randomly on the soil surface in each subplot and within 5–30 min of application, the dishes were collected and placed on ice until they could be transported to the laboratory where they were placed at 5°C for 12 h. The 4 coverslips per dish were placed in phosphate buffer (0.01 M) with 0.05% Tween 80 (buffer-Tween), washed, and colony-forming units (CFU) were counted on the semiselective oatmeal-dodine agar and calculated as CFU/cm^2 (Inglis et al. 1996a).

Droplet deposition was assessed using water-sensitive cards (Teejet Spraying Systems, Wheaton, IL). Cards were set in petri dishes and the dishes were set out at the same time and in the same manner as the coverslips. Cards were collected within 5–30 min of application. Droplet density, area, and size were assessed using a Tracor Northern 8502 Image Analyzer equipped with a Dage 68 video camera for image acquisition. Droplets were analyzed in each of 2 areas (6.2 cm^2) per card; the droplet data from the 2 areas were combined and mean droplet area, width, and density were determined. Total droplet area per card was calculated and converted to percentage of coverage.

Conidial Persistence on Leaves. Immediately after (time 0), and 2, 5, 10, and 15 d following application, 10 grass leaves were collected arbitrarily from each subplot and pooled in plastic bags. Leaves were transported to the laboratory on ice and maintained at 5°C for a maximum of 48 h. All leaves were cut aseptically into pieces ≈1 cm long, and the samples from each subplot were pooled in 5 ml of buffer-Tween, washed, and the number of CFU of *B. bassiana* were assessed. Following washing, the total area of the leaf pieces were determined with a leaf area meter (Model 3100, Li-Cor, Lincoln, NE) and the mean numbers of CFU/cm^2 of leaf area were calculated. Sample leaf areas ranged from 1.2 to 5.0 cm^2. Subplot means were calculated from $\log_{10}$-transformed data, and treatment means were fit to linear models after $\log_{10}$-transformation of the time axis.

Beauveria Associated with Grasshoppers. Grasshoppers were collected in sweepnets from *B. bassiana* and carrier control plots immediately after treatment (time 0), then 5, 10, and 15 d thereafter. Grasshoppers from each plot were placed in cages (44 by 58 by 46 cm) and transported to the laboratory. Within 4–5 h of field collection, 30 and 15 nymphs per plot were selected arbitrarily from the cages containing grasshoppers from the *B. bassiana*-treated and carrier control plots, respectively. Nymphs were placed individually in vials, weighed, and placed at 5°C for a maximum of 48 h. Fifteen nymphs from each of the *B. bassiana*-treated and carrier control plots were homogenized, the homogenate diluted, and the homogenate spread on oatmeal-dodine agar as described previously. The remaining 15 nymphs from the *B. bassiana* sprayed plots were surface-sterilized in ethanol for 1 min, followed by 2 rinses in sterile deionized water before homogenization. The number of CFU per nymph was then determined on oatmeal-dodine agar. To account for variation in the size of nymphs, CFU per nymph were calculated per milligram × the mean weight of nymphs (44.7 mg).

To test the efficacy of the sterilization procedure, 20 laboratory reared, 5th-instar *Melanoplus sanguinipes* (F.) grasshoppers were killed by freezing. Nymphs were weighed, and then sprayed with 100 μl of a suspension of *B. bassiana* conidia in water (10^9 viable conidia per milliliter) using an airbrush (Inglis et al. 1995b). One-half of the nymphs were surface-sterilized in ethanol as above, then all were homogenized individually, and the CFU per insect were enumerated as detailed previously.

Grasshopper Populations, Species and Age Composition. Grasshopper population densities were monitored by counting living grasshoppers in sample areas delimited by 0.25-m^2 sampling frames 1 d before treatment (time 0), and 4, 11, and 15 d after the application of conidia. The sampling frames were open circles of white plastic tubing (0.5 cm diameter) placed on the soil surface in the central sampling zones of each plot (20 per plot).

Mean grasshopper densities per plot were analyzed as a split plot in time (Gomez and Gomez 1984) with 4 levels of block, 3 levels of treatment, and 4 levels of time. A Box correction was used as a conservative test for time (t) and time by treatment interaction (Milliken and Johnson 1984); the Box correction reduces the degrees of freedom for time (t), the time by treatment interaction and the residual error(time) by t-1. Comparisons between means at each sample time were made using the least square means (lsmeans) function of SAS (SAS Institute 1988).

To determine grasshopper species and age composition, 2 samples of 100 sweeps (180°, 38-cm-diameter net) were collected arbitrarily from each plot at time 0, and 4 and 15 d after conidial application. Grasshoppers were returned to the laboratory, frozen, counted, and species and age class determined (Brooks 1958; Otte 1981, 1984; Vickery and Kevan 1983).

Mycosis in Field and Greenhouse Cages. Mycosis was compared in grasshoppers collected from field plots and maintained in field and greenhouse cages; cages consisted of a wood-frame (46 by 61 by 48 cm) covered with fibreglass netting (49 filaments per square centimeter). Grasshoppers were collected from each of the *B. bassiana*-treated and carrier control plots by sweeping within 1–3 h of application (time 0), and 5, 10, and 15 d thereafter. From each plot at each sample time, 100 grasshoppers (primarily melanopline species) were placed into 2 cages. One of the cages was placed adjacent to the field plot, the other was placed in a greenhouse located at the AAFC Research Centre, Lethbridge. All grasshoppers were maintained on a diet of wheat, Norstar, seedlings (10- to 14-d-old in 10-cm-diameter pots), bran, and a variety of grasses and broadleaf plants collected from the field site but not exposed to *B. bassiana*; wheat seedlings, and rangeland grasses and broadleaf plants were replaced daily. Relative humidity, temperature, and solar radiation (400–1,100 nm) were recorded in a cage maintained in the field and in the greenhouse using CR21X microloggers (Campbell, Logan, UT).

Cadavers were removed from cages each morning and placed on moistened filter paper at 25°C in the dark; those that produced hyphal growth of *B. bassiana* were noted. Grasshoppers surviving for 12 d were killed by freezing and similarly placed on moistened filter paper; freezing grasshoppers for short periods has no effect on *B. bassiana* (Inglis et al. 1996c). Nymphs that died and subsequently produced *B. bassiana* hyphae were considered to have died from mycosis; those not producing hyphae were classed as *other mortality*. None of the nymphs that died within 2 d of placement in cages (1–21%) were colonized by *B. bassiana*; these were considered to have died from mechanical damage by sweeping or from natural causes, and they were excluded from subsequent analyses. At each sample date, comparisons of disease progress between field and greenhouse cages were conducted as split plots in time with cage type nested within blocks. The prevalence of final mycosis (12 d) was compared by sample date as a split plot (cage type nested in blocks) with 4 levels of block, 2 levels of cage, and 4 levels of sample time. A Box correction was used in both analyses. When the *F* test for the treatment by time interaction was significant, means were compared using the lsmeans function of SAS (SAS Institute 1988).

Effect of Temperature and Sunlight. Disease was compared in grasshoppers placed in different cages at the field site. The 3 cage treatments included the following: (1) exposed to full spectrum sunlight, (2) shaded from sunlight by a black plastic screen, and (3) protected from UVB radiation by a UVB absorbing plastic film (<355 nm; Dura Film 3, AT Plastics, Edmonton, AB). Plastic screens (1.8 by 3.0 m) were attached to a wood frame. The front of the frame (facing south) was situated 0.9 m above the soil surface and the back of the frame was 1.2 m above the soil; this arrangement shaded the cages for most of day (≈0900–1700 hours) and had a minimal effect on air movement.

Grasshoppers were collected from each of the 4, *B. bassiana* sprayed plots (time 0) by sweeping. Grasshoppers from individual field plots were allocated to 3 cages (100 grasshoppers per cage) and cages were arranged adjacent to the field plots (rangeland site) as a randomized complete block design with 4 blocks, each containing 3 cage treatments per block. Grasshoppers were maintained on a diet of wheat seedlings and rangeland grasses, cadavers were removed daily and placed on moistened filter paper as detailed previously. Throughout the experiment, conditions of light, temperature, and relative humidity were recorded within each of the cage types with a CR21X micrologger.

In a parallel experiment, *M. sanguinipes* nymphs (F_1 laboratory generation) were externally inoculated with *B. bassiana* as described by Inglis et al. (1996d). Nymphs were hatched, reared on a diet of bran and wheat leaves; 3rd instars were collected individually in sterile 20-ml glass vials, and a lettuce disk (5 mm diameter) treated with conidia (0.5 μl) was suspended ≈2 cm into each vial. The inoculation procedure took ≈4 h (n = 1,644 nymphs). Nymphs were allowed an additional 1 h to ingest the disk, and those that molted or that did not consume the entire disk were removed from the experiment. Inoculated nymphs were allocated to 12 cages and cages were placed in a field of crested wheatgrass, *Agropyron cristatum* L., at the AAFC Research Centre, Lethbridge (Lethbridge site). Cages were arranged as randomized complete block design with 3 cage treatments per block.

At both sites, conidial survival was measured on wheat leaves. At the rangeland site, 10- to 14-d-old wheat seedlings in pots (10 cm diameter) were placed in rangeland plots before conidial applica-

tion. Within 5–15 min of application, wheat plants were moved to locations on the soil surface under each UVB screen or adjacent to it in an exposed position (≈5 m distant to prevent any shading). At the Lethbridge site, conidia in water were applied to wheat seedlings using an airbrush (Inglis et al. 1995b). The water carrier was allowed to dry for 15 min, and the plants were located adjacent to each of the cages. Wheat leaves, particularly those in the exposed and UVB protected environments, were subject to clipping by resident grasshoppers at the Lethbridge site. Therefore, pots exposed to full sunlight were transferred to cages to protect them 3 d after conidial application.

At time 0, and 2, 5, and 10 d after conidial application, 10 leaf segments were collected from each pot. At later sampling times, care was taken to sample older leaves and to use segments near the leaf tips. Populations of viable *B. bassiana* conidia were quantified using the wash method as described previously. Conidial populations on leaves were calculated as $\log_{10}$ CFU/cm^2, and were analyzed as a split plot in time with 4 levels of block, 4 levels of environment and 4 levels of time. A Box correction was used for the time and the time by environment interactions. Comparisons between means at each sample time were made using the Tukey test (α = 0.05).

Results

Field Efficacy. *Conidial Deposition.* From coverslips and leaves sprayed with *B. bassiana*, 2.1 × 10^4 (SE = 0.11 × 10^4) and 1.7 × 10^4 (SE = 0.26 × 10^4) CFU/cm^2 were recovered, respectively. No conidia were recovered from leaves or coverslips collected from the carrier control plots. The density of droplets on water-sensitive cards were similar (t = −2.4, df = 6, P = 0.06) between the *B. bassiana* (42.5 droplets/cm^2, SE = 3.0) and carrier control (51.9 droplets/cm^2, SE = 2.4) treatments. Droplet coverage was also similar (t = −1.8, df = 6, P = 0.13) between the *B. bassiana* (15.4%, SE = 1.1) and carrier control (19.9%, SE = 2.3) treatments.

Conidial Persistence on Leaves. Conditions were hot and sunny, and 8 periods of precipitation (≤10 mm per event) were recorded during the course of the experiment (Fig. 1). Conidial survival was poor and populations declined logarithmically over time (Fig. 2).

Beauveria Associated with Grasshoppers. From grasshoppers inoculated with *B. bassiana* in the laboratory, 1.3 × 10^2 (SE = 0.73 × 10^2) CFU per nymph were recovered from those submerged in ethanol for 1 min compared with 1.6 × 10^6 (SE = 0.13 × 10^6) CFU per nymph from unsterilized nymphs, representing >99.9% reduction in conidial populations caused by the sterilization treatment.

Beauveria bassiana was recovered from all nymphs collected from field plots sprayed with co-

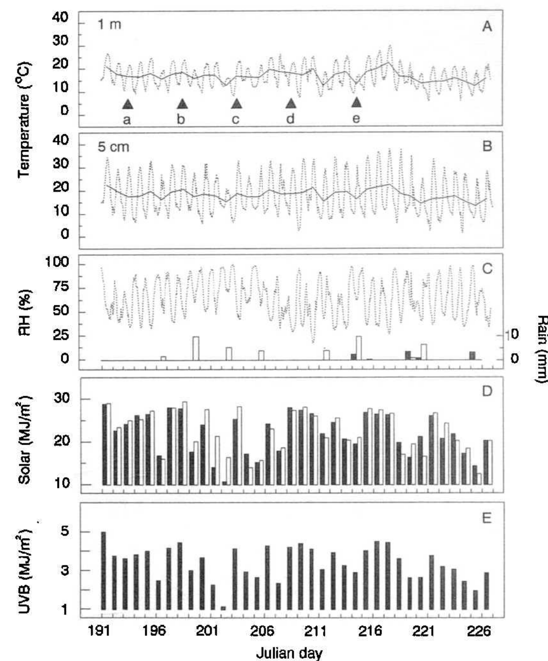

Fig. 1. Weather data from the field experiments (10 July to 14 August 1995). Hourly (dotted lines) and mean (solid lines) temperatures at heights of 1 m (A) and 5 cm (B), and hourly relative humidity (C) were recorded at Lethbridge. Arrows (▲) represent times of conidial application or sample collection (days). (a) Conidia application in rangeland and collection time 0; (b) collection time 5 d; (c) collection time 10 d; (d) collection time 15 d; (e) laboratory inoculation of *M. sanguinipes* nymphs and placement in field cages. Daily precipitation (C; histograms) were recorded at the rangeland (open bars) and Lethbridge (solid bars) field sites. Total daily solar radiation (D; 300–2,800 nm) was recorded at Lethbridge (solid bars) and Vauxhall (open bars). Daily UVB radiation (E) was measured at Lethbridge.

nidia, regardless of whether they were surface-sterilized or not. However, substantially less (t = 38.4, df = 6, P < 0.001) CFU were recovered from surface-sterilized (4.3 × 10^2 CFU per nymphs, SE = 0.87 × 10^2) than from unsterilized nymphs (6.7 × 10^3 CFU per nymph, SE = 0.15 × 10^3) collected immediately after conidial application. Conidial populations recovered from unsterilized grasshopper nymphs declined logarithmically over time (Fig. 2). The slope of conidial persistence on nymphs (-2.82, SE = 0.26) was similar to that on grass leaves (-2.92, SE = 0.30). After the initial sample time, small populations of *B. bassiana* (<5 CFU per nymph) were recovered from surface-sterilized nymphs at subsequent sample times. Low numbers of *B. bassiana* (<30 CFU per nymph) were recovered from nymphs collected from the carrier control plots at all sample times.

Grasshopper Populations, Species, and Age Composition. At the time of application, mean population densities ranged from 7 to 9 grasshoppers per square meter, and 67% of the individuals

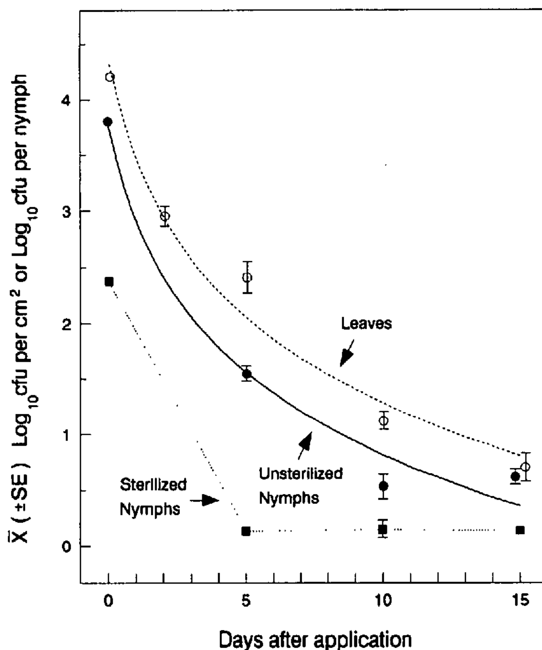

Fig. 2. Populations of *B. bassiana* recovered from grass leaves and grasshopper nymphs collected from field plots sprayed with conidia. From grass leaves (○), populations were quantified as $\log_{10}$ CFU/cm^2 of leaf area. From grasshopper nymphs, populations were quantified as log CFU per nymph; to account for variation in nymph size, weights were standardized to 44.7 mg. Nymphs were either unsterilized (●) or sterilized (■) before homogenization. Vertical lines represent standard errors of means (n = 4); to avoid superimposition of the standard error bars, some means are offset along the x-axis. For leaves and unsterilized nymphs, data were fit to linear equations after log-transformation of conidial population and time data. For leaves, the coefficient of determination (r^2) was 0.98 and the equation used to describe the relationship was $\log_{10}$ CFU/cm^2 = 4.32 − 2.92 [$\log_{10}$ (days + 1)]; SE were 0.21 and 0.26 for the y-intercept and slope, respectively. For unsterilized nymphs, the r^2 was 0.98 and the equation used to describe the relationship was $\log_{10}$ CFU per nymph = 3.76 − 2.82 [$\log_{10}$(days + 1)]; SE were 0.26 and 0.30 for the y-intercept and slope, respectively.

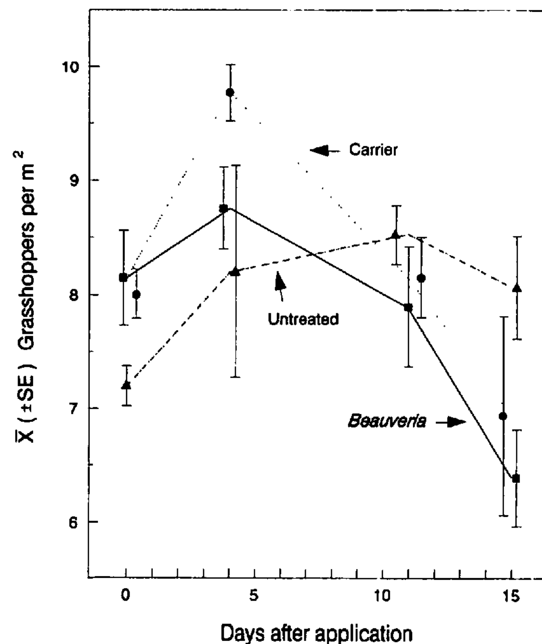

Fig. 3. Grasshopper densities (grasshoppers per square meter) for the *B. bassiana* (■, *Beauveria*), carrier control (●, carrier), and unsprayed treatments (▲, untreated) taken before application of conidia (time 0), and 2, 11, and 15 d after conidial application. Vertical lines represent standard errors of means (n = 4). To avoid superimposition of standard error bars, means are slightly offset along the x-axis.

(n = 605) were 3rd or 4th instars. After 15 d, 31% of the grasshoppers collected (n = 835) were adults. The application of *B. bassiana* did not affect (F = 2.3; df = 2, 9; P = 0.16) densities of grasshoppers observed in the sampling frames relative to the other treatments (Fig. 3). The predominant grasshoppers collected were *M. infantilis* Scudder (67%, n = 1,509); *Aeropedellus clavatus* (Thomas) (7%, n = 153); *M. sanguinipes* (5%, n = 111); *Ageneotettix deorum* (Scudder) (4%, n = 91); *M. gladstoni* Scudder (4%, n = 84); *Philibostroma quadrimaculatum* (Thomas) (3%, n=70); *Phoetaliotes nebrascensis* (Thomas) (3%, n = 64); and *M. packardii* Scudder (3%, n = 59). There were no conspicuous shifts in species composition of dominant grasshopper taxa collected in sweepnets between the *B. bassiana* and control treatments.

Mycosis in Field and Greenhouse Cages. In total, 6,400 grasshoppers were collected from *B. bassiana*-treated and carrier control plots over the 15-d sampling period. In nymphs collected from the carrier control plots, 0–7% mycosis was observed after 12 d; we judged this to be negligible and excluded this treatment from all subsequent analyses of mycosis. Disease progressed more rapidly (F = 12.4–189; df = 1, 6; $P \leq 0.013$) and attained a higher prevalence of final mycosis (F = 226; df = 1, 6; $P < 0.001$) in nymphs from *B. bassiana* plots placed in greenhouse than in field cages (Fig. 4). In the greenhouse cages, disease levels at 12 d deceased (F = 7.5; df = 3, 9; P = 0.008) with sample time; less mycosis ($P \leq 0.02$) was observed in grasshoppers collected 10 and 15 d after conidial application. However, mycosis first occurred 3–4 d after placement of the nymphs in greenhouse cages regardless of the collection time. Disregarding grasshoppers that died within 2 d of placement in cages, mortality not attributed to *B. bassiana* ranged from 1.1 (SE = 1.1) to 19.7 % (SE = 2.6) by 12 d. Non-*B. bassiana* mortality was not influenced by either sample time (F = 0.66; df = 1, 12; P = 0.43) or cage environment (F = 1.6; df = 1, 3; P = 0.26).

During the day, temperatures in the field and greenhouse cages differed by ≤5°C (Fig. 5A); mean maximum temperatures were 33°C (n = 26

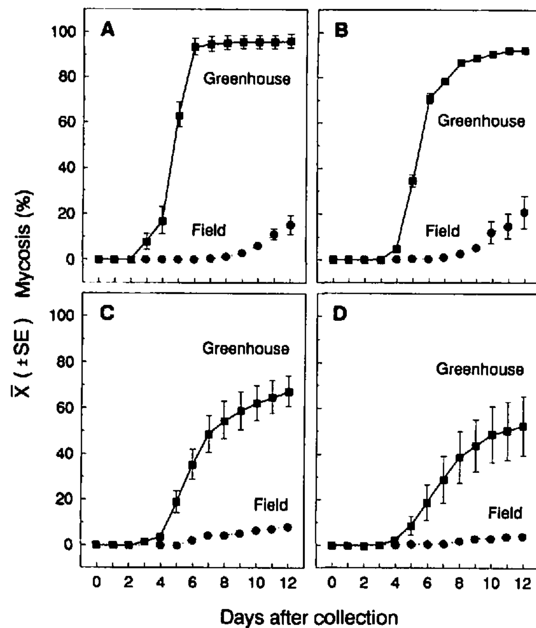

Fig. 4. Disease progress for rangeland grasshoppers collected from *B. bassiana*-treated field plots maintained in greenhouse (■) and field cages (●). Grasshoppers were collected (A) immediately after conidial application, (B) 5 d after application, (C) 10 d after application, and (D) 15 d after application. Vertical lines represent standard errors of means (n = 4).

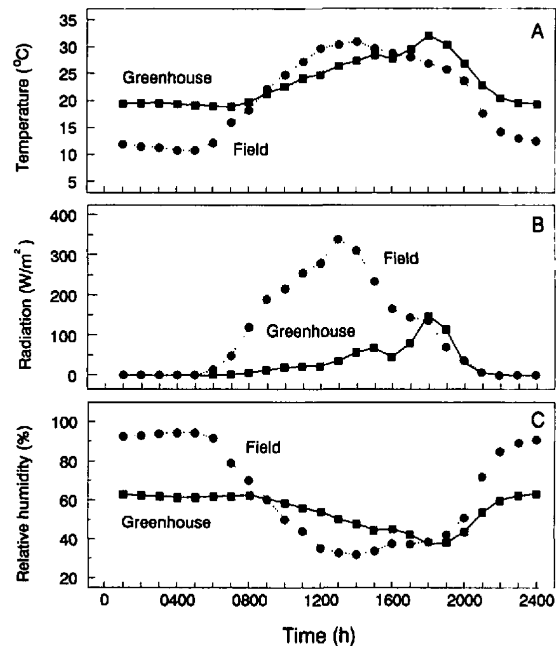

Fig. 5. Mean hourly conditions of temperature, solar radiation, and relative humidity in cages maintained in the greenhouse (■) and field environments (●).

d). At night, temperatures in the greenhouse cages were up to 8°C higher on average than in the field cages; mean minimum temperatures were 19°C in the greenhouse and 9°C in the field. Shades were drawn in the greenhouse to prevent overheating and 74% less solar radiation was recorded in the greenhouse than in the field cages (Fig. 5B). During the day, relative humidities in the 2 types of cages differed by <19% (Fig. 5C) but nighttime humidities in the greenhouse cages were substantially (24 to 33%) lower than in the field cages. There were minimal differences in temperature (≤3°C) and relative humidity (≤5%) between the inside and outside of the field cages. However, the cage mesh caused considerable shading, and 57% less solar radiation was recorded in the cage.

Effect of Temperature and Sunlight. Grasshoppers were observed to congregate in areas of high sunlight (basking behavior) in the exposed and UVB-protected cages. Congregation of grasshoppers was not observed in the shaded cages. At both the rangeland and Lethbridge sites, the rate of disease development (F = 146–101; df = 2, 9; P < 0.001) and the prevalence of final mycosis (F = 109–3,340; df = 2, 9; P < 0.001) differed among the 3 cage environments (Fig. 6); disease development was more rapid (P < 0.05) and the prevalence of final mycosis (83–89%) was higher (P < 0.001) in the shaded than in the exposed (0–15%) and UVB-protected (1–43%) field cages in both

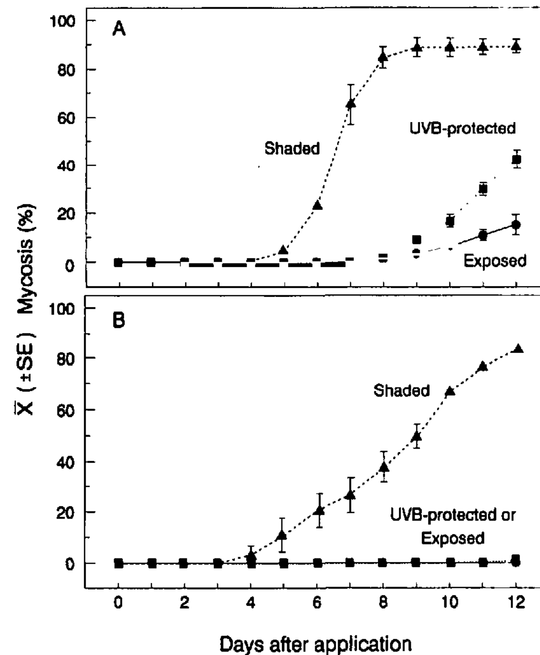

Fig. 6. Disease progress for grasshoppers maintained in field cages that were shaded (▲, shaded), exposed to UVB-filtered sunlight (■, UVB-protected), or to full spectrum sunlight (●, exposed). (A) Rangeland grasshoppers collected from field plots sprayed with *B. bassiana* conidia, and (B) *M. sanguinipes* nymphs inoculated with conidia. Vertical lines represent standard errors of means (n = 4).

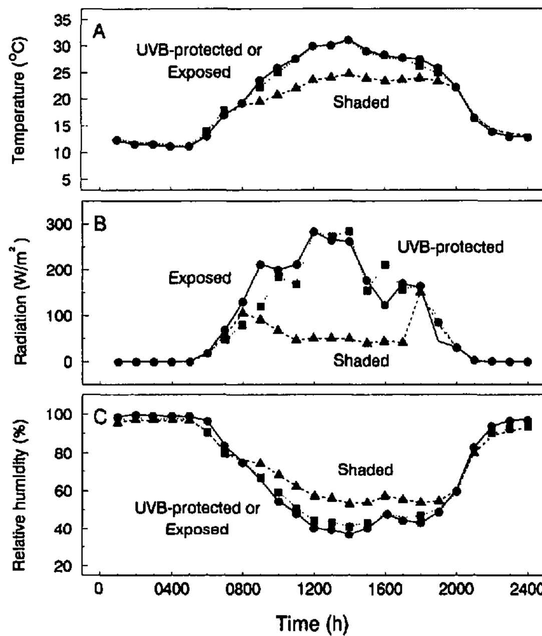

Fig. 7. Mean hourly conditions of (A) temperature, (B) solar radiation, and (C) relative humidity in cages at the rangeland field site. Cages were shaded (▲, shaded), exposed to UVB-filtered sunlight (■, UVB-protected), or exposed to full spectrum sunlight (●, exposed).

trials. At the rangeland (Fig. 6A) but not the Lethbridge (Fig. 6B) site, substantial mycosis (≥15%) was observed in grasshoppers placed in the UVB-protected and exposed field cages. Mycosis was first observed in these cages 8 d after inoculation during a relatively cool overcast period. At the end of the experimental period, more grasshoppers ($P < 0.001$) had died of mycosis in the UVB-protected (42.5%, SE = 3.9) than in the exposed (15.2%, SE = 4.0) cages. The incidence of other mortality ranged from 10.0% (SE = 2.8) to 17.7% (SE = 7.8) at the rangeland site, and from 1.5% (SE = 0.06) to 10.7% (SE = 1.3) at the Lethbridge site.

Temperatures, relative humidities, and solar radiation (>400 nm) were similar in the exposed and UVB-protected cages at the rangeland (Fig. 7) and Lethbridge (data not presented) sites. Conditions were generally hot and sunny; temperatures reached or exceeded 35°C in the exposed and UVB-protected cages for relatively short periods during 5 d in each trial. In contrast to exposed and UVB-protected cages, relative humidities were up to 17% higher, temperatures were up to 6°C cooler, and visible light was reduced by 59–80% in the shaded cages during the day (Fig. 7).

The cage environment significantly affected ($F = 6.8$–21.2; df = 1–2, 7–8; $P \leq 0.035$) conidial survival (Table 1). At both sites, more CFU ($P \leq 0.05$) were recovered from wheat leaves placed under the UVB-screen than those exposed to full spectrum sunlight. At the Lethbridge site, there was no difference ($P \leq 0.05$) in numbers of CFU recovered from the shaded and UVB-protected environments immediately after, 2 and 5 d after conidial application; at the 10 d sample time, most of the leaves under the UVB-film had been consumed by resident grasshoppers.

Discussion

Field Efficacy and Environment. Despite the deposition of substantial quantities of conidia onto grasshoppers (Fig. 2), no reductions were detected in field populations (Fig. 3). Although some disease was observed in grasshoppers maintained in cages adjacent to the field plots, substantially higher levels of mycosis were observed in grasshoppers maintained in greenhouse cages (Fig. 4). The prev-

Table 1. Populations (mean ± SE) of *B. bassiana* conidia ($\log_{10}$CFU/cm²) on wheat leaves

Environment	Days after application			
	0	2	5	10
		Rangeland site		
Shaded	—	—	—	—
UVB	4.17 ± 0.09	4.23 ± 0.06a	3.46 ± 0.10a	1.95 ± 0.30a
Exposed	4.41 ± 0.16	3.56 ± 0.08b	2.88 ± 0.09b	0.42 ± 0.42b
F	1.8	41.8	18.1	8.7
df	1, 6	1, 6	1, 6	1, 6
P	0.23	0.001	0.005	0.026
		Lethbridge site		
Shaded	5.24 ± 0.03	4.46 ± 0.07a	3.42 ± 0.12a	2.40 ± 0.23a
UVB	5.32 ± 0.07	4.10 ± 0.17a	2.99 ± 0.07a	—[a]
Exposed	5.37 ± 0.07	3.44 ± 0.10b[b]	1.83 ± 0.30b	0.0b
F	0.96	17.9	18.2	110.3
df	2, 9	2, 9	2, 9	1, 6
P	0.42	0.001	0.001	0.001

Wheat plants were placed under black plastic (shaded), placed under a UVB absorbing film (UVB), or exposed to full spectrum solar radiation (exposed). Means at each site and within each column that are not followed by the same letter are significantly different from each other according to the Tukey test ($\alpha = 0.05$).

[a] Wheat leaves were extensively clipped by resident grasshoppers.

[b] Wheat plants were transferred to cages to prevent foraging by indigenous grasshoppers.

alence of mycosis in greenhouse cages decreased with sample time but the onset of disease was always 3–4 d after the placement of nymphs in cages, regardless of when the grasshoppers were collected; this observation agrees with earlier reports (Johnson and Goettel 1993, Inglis et al. 1996a). Predisposition of insects to infection by entomopathogens has been reported (Steinhaus 1958, Ferron 1971, Donegan and Lighthart 1989, Fargues et al. 1991) and grasshoppers were exposed to a variety of factors that may have caused stress (e.g., collection in sweepnets, confinement in cages or altered diet). However, with exception of cage environment, they were treated similarly, suggesting that the differences observed in susceptibility of grasshoppers in the 2 cage types were caused by environmental conditions and not by stress independent of environment. Although our results indicate that a conducive environment is essential for disease development, the mechanism(s) by which environment influences mycosis was uncertain.

Temperature and Sunlight. Conditions of temperature, light exposure, and relative humidity differed between the field and greenhouse cage environments (Fig. 5). Relative humidity has a negligible effect on infection of grasshoppers by *B. bassiana* (Marcandier and Khachatourians 1987), and mean hourly temperatures were only slightly cooler in the greenhouse for most of the day. Nighttime temperatures were higher (≈8°C) and light levels were substantially reduced (≈74%) in the greenhouse cages, and these factors could have adversely affected disease development in grasshoppers maintained in the field cages. Although no reductions were detected in field populations of acridids, we observed some disease (15–21%) in grasshoppers collected within 5 d of conidial application and maintained in cages adjacent to the field plots (Fig. 4). The mesh covering the field cages had a minimal influence on temperature and relative humidity but caused considerable shading (≈55%) further implicating light as a factor influencing the susceptibility of grasshoppers to *B. bassiana*.

Grasshopper Thermoregulation and Conidial Survival. Grasshoppers elevate their body temperature higher than ambient by directly or indirectly intercepting solar radiation (Chappell and Whitman 1990, Heinrich 1993). Given the opportunity, grasshoppers optimized their body temperature (≈38–40°C) by basking, and mycosis (*B. bassiana*) was reduced by 46% in grasshoppers that we allowed to bask for only 1 h/d (Inglis et al. 1996b). We observed that, levels of light were 61–80% less, daytime temperatures were up to 6°C cooler (Fig. 7), and mycosis was substantially greater in the shaded (>80%) than in unshaded field cages (0–15%) (Fig. 6). Temperatures in the unshaded cages reached or exceeded 35°C in only 5 d per trial, and then for relatively short periods of time (generally <4 h/d). Because exposures to 35°C for ≤4 h/d has no effect on mycosis (Inglis et al. 1996b), cage temperatures alone cannot explain the differences in disease between the 2 cage environments. Furthermore, basking behavior (e.g., congregation in areas of intense sunlight) was only observed in grasshoppers in the unshaded cages. The detrimental effects of grasshopper thermoregulation on mycosis is consistent with observations of successful suppression of field populations of grasshoppers with *B. bassiana* during cool overcast periods (Johnson and Goettel 1993) but not during hot sunny periods (Inglis et al. 1996a).

Exposure to light, in particular the UVB portion of the solar spectrum, adversely affects the survival of *B. bassiana* conidia in epigeal habitats (Inglis et al. 1993, 1995a), and we observed that conidia deposited on grass leaves were killed rapidly (Fig. 2). Inglis et al. (1996a) observed that the persistence of *B. bassiana* associated with field-collected grasshoppers was similar to that on leaves, an observation substantiated in the current study. However, Inglis et al. (1996a) were unable to distinguish between conidia on the surface of nymphs and blastospores and hyphae in the hemocoel. We compared populations of *B. bassiana* from surface-sterilized (internal CFU) and unsterilized nymphs (internal and external CFU) at various times after conidial application. From surface-sterilized nymphs, *B. bassiana* was recovered in substantial numbers only from grasshoppers collected immediately after conidial application (Fig. 2). Conidia ingested by grasshoppers survive passage through the alimentary tract (Inglis et al. 1996c), and *B. bassiana* CFU recovered from surface-sterilized nymphs immediately after application likely represent conidia deposited onto foliage that were ingested by nymphs. The low numbers of conidia recovered from surface-sterilized grasshoppers at subsequent collection times indicates that the majority of *B. bassiana* conidia deposited on grasshoppers remain exposed on the surface of the integument, and if infection occurred, the fungus did not proliferate in the hemocoel.

To determine the relative importance of grasshopper thermoregulation and the deactivation of conidia by UVB radiation on mycosis, we compared disease development and conidial survival in UVB-protected and shaded environments. Conidial survival was similar between the 2 environments (Table 1), but substantially more disease was observed in the shaded (>80%) than in the UVB-protected (1–43%) cages at both sites (Fig. 6). These observations indicate that the indirect effects of temperature and light on the susceptibility of grasshoppers to *B. bassiana* (i.e., behavioral thermoregulation) had a greater influence on disease development than did the rapid deactivation of conidia by UVB radiation.

Although the ability of grasshoppers to elevate their body temperature influenced mycosis, evidence also suggested that conidial survival had an effect on disease. Conditions of visible light were

similar between the UVB-protected and unshaded environments (i.e., grasshoppers had similar opportunity to thermoregulate) (Fig. 7), but conidial survival was enhanced in the former (Table 1). At the rangeland site, mycosis was substantially higher in the UVB-protected (43%) than in the unshaded (15%) cages, and disease was first observed in both cage types, 8 d after conidial application during a relatively cool, overcast period. These observations suggest that by prolonging conidial survival until conditions are conducive for disease development (i.e., during a period when grasshoppers were incapable of thermoregulation), the field efficacy of *B. bassiana* may be enhanced. Inglis et al. (1995a) demonstrated that conidia formulated in sunscreens survive longer than unprotected conidia in field settings, and it may be possible to use sunscreen formulations to study the relationship between conidial deactivation and disease development.

An understanding of the factors that limit the development of epizootics is imperative if *B. bassiana* is to be used to manage insects. By studying conidial survival and disease development in different environments, we demonstrated that solar radiation and temperature, and not pathogen virulence or host targeting, limited the efficacy of *B. bassiana* against acridids in field settings. The low levels of disease that were observed in unshaded and UVB protected cages is consistent with the ability of grasshoppers to elevate their body temperatures (behavioral thermoregulation). However, conidial deactivation by UVB radiation appeared to influence disease development in 1 of 2 trials. An understanding of the relationship between conidial survival and the ability of grasshoppers to elevate their body temperature is necessary, as is determination of the timing and likelihood of weather conditions that may affect these variables. Furthermore, the profound effect that the cage environment has on mycosis emphasizes that caged insects should not be used for assessing the field efficacy of entomopathogenic Hyphomycetes against grasshoppers, and possibly against many other insects including nontarget arthropods. Ultimately the success of *B. bassiana* against acridids in field settings will depend on the development of biorational strategies that overcome the light and temperature constraints of *B. bassiana*.

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