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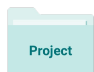

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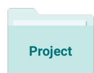

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FIELD AND LABORATORY EVALUATION OF TWO CONIDIAL BATCHES OF *BEAUVERIA BASSIANA* (BALSAMO) VUILLEMIN AGAINST GRASSHOPPERS

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Abstract

The Canadian Entomologist **129**: 171–186 (1997)

The efficacy of two production batches of conidia of *Beauveria bassiana* (Balsamo) Vuillemin that showed differential field efficacy in 1992 (GHA 92) and 1994 (GHA 94) were compared against grasshoppers in the laboratory and field. Conidia of GHA 92 and GHA 94 exhibited good germination (>92%) by 24 h, but the rate of germination was slower for GHA 94 than for GHA 92. Although both conidial batches were highly virulent ($LD_{50} < 6 \times 10^3$ conidia per nymph) against nymphs of *Melanoplus sanguinipes* (Fabricius) (Orthoptera: Acrididae) in the laboratory, GHA 92 was slightly more virulent than GHA 94. Conidia were applied to field populations of grasshoppers in a 1.5% emulsifiable oil-emulsion amended with 4% clay at a volume of 112 L/ha. There were no differences between GHA 92 and GHA 94 in the deposition of spray droplets on water-sensitive papers or of conidia on leaves and coverslips (2.4×10^4 to 4.1×10^4 cfu/cm^2). All grasshopper nymphs collected from plots sprayed with conidia of GHA 92 and GHA 94 were equally infested with *B. bassiana*; conidial populations averaged 3.5×10^3 to 4.3×10^3 cfu/nymph. Conditions were hot, dry, and sunny, and regardless of the batch, persistence of conidia was equally short on both leaves and grasshoppers. Neither treatment of *B. bassiana* significantly reduced field populations nor did either impact differentially on specific grasshopper taxa. However, among grasshoppers collected immediately after conidial application and maintained in cages in the greenhouse, over 80% died of infection with *B. bassiana*. For both conidial treatments, the prevalence of disease in caged grasshoppers decreased with the sampling date but the onset of mycosis always occurred 3–4 days after collection. This study indicates that environmental conditions in the field and not pathogen virulence or targeting were responsible for the poor efficacy of *B. bassiana* against grasshoppers.

Inglis, G.D., D.L. Johnson et M.S. Goettel. 1997. Évaluation en laboratoire et sur le terrain de deux lots de conidies de *Beauveria bassiana* dans la lutte contre les sauterelles. *The Canadian Entomologist* **129**: 171–186.

Résumé

Les effets de deux lots de conidies de *Beauveria bassiana* (Balsamo) Vuillemin qui ont eu une efficacité différente sur le terrain en 1992 (GHA 1992) et en 1994 (GHA 94) ont été comparés en laboratoire et sur le terrain dans la lutte contre les sauterelles. Les conidies de GHA 92 et de GHA 94 avaient des taux de germination élevés (>92%) après 24 h, mais la germination était plus lente dans le cas de GHA 94 que dans le cas de GHA 92. Bien que les deux lots de conidies se soient avérés très virulents ($LD_{50} < 6 \times 10^3$ par larvae) pour les larves de *Melanoplus sanguinipes* (Fabricius) (Orthoptera: Acrididae) en laboratoire, le GHA 92 était légèrement plus virulent que le GHA 94. Une émulsion à l'huile émulsifiable 1,5% de conidies additionnée de 4% d'argile a été vaporisée sur le terrain à raison de 112 L/ha. Il n'y avait pas de différences entre les lots GHA 92 et GHA 94 quant à la répartition des gouttelettes d'émulsion sur des papiers sensibles à l'eau ou des conidies sur des feuilles ou des lamelles ($2,4 \times 10^4$ à $4,1 \times 10^4$ cfu/cm^2). Toutes les larves de sauterelles recueillies dans les champs vaporisés

de conidies de GHA 92 et GHA 94 étaient également infestées de *B. bassiana*; les populations de conidies contenaient en moyenne de $3,5 \times 10^3$ à $4,3 \times 10^3$ cfu/larve. Les conditions étaient chaudes, sèches et ensoleillées et la persistance des conidies des deux lots était de courte durée sur les feuilles et sur les sauterelles. Ni l'un ni l'autre des traitements n'a entraîné de réduction significative des populations de sauterelles et leurs effets sur des taxons spécifiques de sauterelles ont été sensiblement les mêmes. Cependant, parmi les sauterelles recueillis immédiatement après l'application du traitement et gardés en serre dans des cages, plus de 80% sont morts à la suite de l'infection par le champignon. Dans le cas des deux traitements, la fréquence des infections chez les sauterelles en cage diminuait en fonction de la date de l'échantillonnage, mais le déclenchement de la mycose avait toujours lieu de 3 à 4 jours après l'échantillonnage. Les résultats de l'étude indiquent que ce sont les conditions climatiques sur le terrain et non la virulence du pathogène ou l'hôte visé qui sont responsables de l'inefficacité des traitements à base de *B. bassiana* dans la lutte contre les sauterelles.

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Introduction

Control strategies for grasshoppers and locusts rely almost exclusively on the use of chemical insecticides. Recognition of the deleterious effects of pesticides have prompted the development of alternative, less obtrusive management strategies such as the use of microbial control agents (Goettel et al. 1995). The entomopathogenic fungus, *Beauveria bassiana* (Balsamo) Vuillemin, has shown potential for the management of insects (Feng et al. 1994), and its pathogenicity has been demonstrated in a field environment against grasshoppers (Johnson and Goettel 1993). Although application of *B. bassiana* has successfully reduced acridid populations in some field experiments, field efficacy is variable (Johnson et al. 1992; Lobo-Lima et al. 1992; Johnson and Goettel 1993). Mycotech Corporation Inc. (Butte, MT) is developing a strain of *B. bassiana* (GHA) as a commercial microbial control agent against grasshoppers. Conidia of *B. bassiana* (GHA 92) produced in 1992 and aerially applied in oil were effective against grasshoppers, whereas conidia of the same genotype produced in 1994 (GHA 94) and similarly applied were ineffective (R.N. Foster et al., unpublished; C.A. Bradley, pers. comm.). The most obvious possibilities for the decreased efficacy of GHA 94 were loss of virulence, inadequate targeting of grasshoppers with conidia, or both. The objective of our study, in July 1994, was to determine whether the observed differences in efficacy between GHA 92 and GHA 94 were caused by changes in the pathogen or by other aspects of pathogen–grasshopper–environment interaction.

Materials and Methods

Laboratory Assays. Inoculum production. The strain of *B. bassiana* used (GHA) is pathogenic to the migratory grasshopper, *Melanoplus sanguinipes* (Fabricius) (Orthoptera: Acrididae). Conidia were produced using solid-substrate technology (Bradley et al. 1992) and those produced in 1992 (GHA 92; batch 921114) and 1994 (GHA 94; batch 940302) were dried (< 10% moisture) and maintained at 0–5°C.

Viability assessments. Dry conidia from both production batches, and a GHA standard (GHA STD) conidial batch stored at −10°C and known to be highly virulent against grasshoppers (Inglis et al. 1996*a*), were suspended in sterile deionized water. The conidia were vigorously mixed with a micropestle to facilitate suspension. Conidial concentrations were measured using a hemocytometer, adjusted to 1×10^6 conidia/mL, and 100-µL aliquots from each suspension were spread on potato dextrose agar (PDA; Difco) amended with 0.005% Benlate, 0.04% Pen G, and 0.1% Streptomycin. After 6, 12, 18, and 24 h at 25 ± 1°C, conidia were fixed with lactophenol and germination rates determined by examination of a minimum of 500 conidia from each of three replicate cultures. Conidia were

considered viable if germ-tubes were greater than 3 μm in length. The experiment was conducted three times ($n = 3$ cultures per time) and data from the three trials were combined after testing for homogeneity of variance using Bartlett's test (Steel and Torrie 1980). Germination percentages were compared among GHA strains over time as a split-plot in time (Gomez and Gomez 1984) with nine levels of replicate, three levels of conidial batch, and four levels of time. A Box correction was used as a conservative test for time (t) and time–treatment interactions (Milliken and Johnson 1984); the Box correction reduces the degrees of freedom for time (t), the time–treatment interactions, and the residual error(time) by t–1. Comparisons among means at each sample time were made using the least-square means function of SAS (SAS Institute Inc. 1988).

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Virulence assessments. Nymphs of a non-diapause strain of *M. sanguinipes* were reared on a diet of bran and wheat seedlings. Third-instar nymphs were individually collected and inoculated according to Inglis et al. (1996*b*). A lettuce disk (5 mm diameter) treated with conidia (0.5 μL) was suspended approximately 2 cm into a vial containing a third-instar nymph. Nymphs were allowed up to 4 h to ingest the disks, and nymphs that molted or did not consume the entire disk during this period were removed from the experiment. Conidial concentrations were adjusted so that 0.5 μL of the oil carrier contained either 3, 3.5, 4.0, 4.5, 5.0, or 5.5 log viable conidia; the control treatment consisted of oil alone. Following the ingestion period, 25 nymphs per treatment were transferred to plexiglass containers (21 by 28 by 15 cm) and maintained in a controlled environment chamber (CEC) under a 16L:8D photoperiod at a 25°C day : 20°C night temperature regime. Nymphs were fed wheat leaves twice daily. Mean hourly ambient and within-cage temperatures and relative humidities were recorded with a CR21X micrologger (Campbell Scientific Co.). Average daily temperatures in the cages were within 1°C of ambient during the dark period but rose approximately 3°C higher than ambient during the light period. Average daily relative humidities within the cages ranged from 26.3 to 33.7%. Humidity was within 4% of ambient for most of the day but rose up to 9% higher than ambient immediately after placement of the wheat leaves in the cages.

Dead nymphs were removed from the cages at 8- to 12-h intervals and cadavers were placed on moistened filter paper at 25°C in the dark. Those cadavers that produced hyphal growth of *B. bassiana* were considered to have died from mycosis; those not producing hyphal growth were recorded as "other mortality". Nymphs that survived 14 days were killed by freezing and also placed on moistened filter paper [freezing has no effect on *B. bassiana* (Inglis et al. 1996*a*)]. The experiment was repeated with the exception of the log 5.5 dose. The total number of nymphs used was 859, consisting of 42–49 nymphs per treatment–dose combination.

The prevalence of disease at 8 and 14 days was analyzed as a randomized complete block design (RCBD) with two levels of block, three levels of conidial batch, and five levels of dose; least-square means was used to compare the results averaged over dose. In the absence of a block effect ($P \geq 0.05$), data were combined and probit-transformed regressions of mycosis at 8 and 14 days by log-dose were fitted by maximum likelihood using the S108 program (Agriculture and Agri-Food Canada); a normal distribution was assumed. The program also tested for goodness of fit, estimated effective $dose_{50}$ with 95% fiducial limits, and parallelism and a common intercept.

Field Trial. Application. The field experiment was conducted 7 km northwest of Barnwell, Alberta. The field site was native short-grass prairie rangeland. Dominant species were spear grass (*Stipa comata* Trin. & Rupr.), blue grama [*Bouteloua gracilis* (H.B.K.) Lag. ex Steud.], june grass (*Koeleria gracilis* Pers.) with subdominant low-growing broadleaf plants and other grasses.

Treatments were conidia of GHA 92, of GHA 94, a carrier control, and an untreated control, arranged in a RCBD with two blocks per treatment; each block consisted of four

2-ha plots (141 by 141 m) separated by a minimum distance of 100 m. To eliminate contamination of the sprayer, the carrier control treatment was applied before the conidial treatments. Five 4-m^2 square subplots were placed within each plot. Four of the subplots were inside the plot boundary, 30 m diagonally from the corners; the fifth subplot was at the center of the plot.

Conidia (2.5×10^{11} conidia/mL) were suspended in a 1.5% (v/v) emulsifiable oil-emulsion amended with 4% clay (w/v) for a target spore concentration of 2.5×10^{13} conidia/ha. Treatments were applied at a rate of 112 L/ha (207 kPa) with a tractor-mounted Hardi sprayer traveling at 12 km/h. The boom was 9.5 m in length equipped with 20 TeeJet 110-2R flat-fan nozzles (50-cm spacing), adjusted to a height of 45 cm. Conidia were applied on the morning of 12 July 1994.

Application rates. Water-sensitive papers (TeeJet Spraying Systems Co.) were placed on the soil surface in each of the four peripheral subplots with the exception of the untreated plots. Cards were collected 5–15 min after application. Droplet density, area, and size were assessed on the cards using a Tracor Northern 8502 Image Analyzer. Standard errors of the mean (SE) were calculated for individual means.

Conidial deposition on coverslips was quantified. Five coverslips (13 mm diameter) were positioned in a petri dish, and one dish was placed in each of the four peripheral subplots. The dishes were collected within 15 min of application, transported to the laboratory on ice, and maintained at 5°C for 12 h. The five coverslips per dish were aseptically placed in 5 mL of 0.01 *M* phosphate buffer with 0.01% Tween 80 (buffer-Tween) in 20-mL vials and washed at ambient temperature for 2 h on a rotary shaker at 300 rpm. After shaking, conidial densities per square centimetre were measured on a semi-selective oatmeal-dodine medium (Inglis et al. 1993). Subplot and treatment means were calculated from untransformed data.

Weather conditions. Mean hourly total solar radiation (300–2800 nm), temperature, and relative humidity were recorded at weather stations located at Lethbridge and Vauxhall; ultraviolet B (UV-B; 280–320 nm) radiation was also measured at Lethbridge. Temperatures and relative humidities recorded at the two weather stations were similar, and because the field site was located between them (36 km W and 30 km NE from Lethbridge, Vauxhall, respectively), it was concluded that the data recorded at Lethbridge were representative of the field site. Daily precipitation was recorded 15 km SE from the field site by the Taber Sugar Company.

Conidial persistence on leaves. Twenty grass leaves were collected immediately after, and 2, 5, 10, and 15 days post-application from each of the subplots, and transported to the laboratory in plastic bags on ice. Leaves were aseptically cut into pieces approximately 1 cm long, bulked by subplot in 5 mL of buffer-Tween, and conidia of *B. bassiana* enumerated on oatmeal-dodine agar (Inglis et al. 1993); representative colonies were examined microscopically to confirm the identity of *B. bassiana*. The total area of the washed leaf pieces, determined with a leaf area meter (Model 3100, Li-Cor Inc., Lincoln, NE, USA), ranged from 3.6 to 12.9 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 data. Untransformed means are presented in the text.

Conidial populations on nymphs. Samples of approximately 150 grasshoppers per plot were swept from plots immediately after, and 2, 5, 10, and 15 days post-application. Nymphs were transferred to cylindrical cages (45.5 cm high by 14.5 cm diameter) and transported to the laboratory. With the exception of the first sample time, cages were separated from each other by a plastic barrier to prevent cross-contamination. Twenty, third- and fourth-instar nymphs were placed individually in sterile glass vials within 4–5 h of field collection, weighed, and maintained at 5°C for a maximum of 48 h. Individual nymphs were

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homogenized in 1 mL of buffer-Tween, the suspension diluted, colony-forming units (cfu) of *B. bassiana* enumerated on oatmeal-dodine agar. The mean weight of nymphs was 48 mg, and cfu populations per nymph were calculated per mg × 48 mg to account for variation in nymphal size.

Mycosis in field-collected grasshoppers. From the transport cages, 100 arbitrarily selected grasshoppers were transferred to wire-mesh cages (51 by 23 by 23 cm) randomly placed in a greenhouse. Grasshoppers were maintained on a diet of wheat seedlings, bran, and a variety of grasses and broadleaf plants collected from the field site (not exposed to *B. bassiana*). Cadavers were removed daily and placed on moistened filter paper. Temperature and relative humidity in the greenhouse were measured with a hygrothermograph. Shades were drawn in the greenhouse to provide conditions of diffuse light. Daily mean temperatures and relative humidities (± standard deviations) in the greenhouse were 3.7 ± 0.52°C and 18.9 ± 1.8% higher on average than the field environment. At each sample time, disease progress among conidial treatments was compared as a split-plot with two levels of replicate and conidial batch, and 10 levels of time. In addition, the prevalence of maximum mycosis at 10 days was compared over sample time as a split-plot with two levels of replicate and conidial batch, and five levels of sample time.

Grasshopper populations, species, and composition. Grasshopper species and age composition (Brooks 1958; Otte 1981, 1984; Pfadt 1988; Vickery and Kevan 1985) were assessed from 200 random sweeps (180°, 38-cm-diameter net) in two transects per plot on each sampling date. Grasshopper population densities were monitored by counting living grasshoppers in permanently situated, 0.25-m^2 sampling frames, on the day of application, and 1, 9, and 15 days after the application of conidia of *B. bassiana*. Two types of frames were used (open squares and circles). The sampling frames were placed on the soil surface in the central sampling zones of the plots prior to the beginning of the experiment in three marked transects (20 rings, 18 squares, and 20 rings, respectively) per plot. Rings and squares gave similar counts ($r = 0.758$, $P < 0.001$); the average ratio of mean counts in rings to mean counts in squares was 1.0 (SE = 0.057).

Logarithmically transformed densities of grasshoppers in both types of frames were analyzed as a split-plot in time with four levels of treatment, two levels of block, and four levels of time. In addition, reductions in treated plots were adjusted against reductions in the untreated plots relative to population densities at day 0 (Abbott 1925; Schaalje et al. 1986). To determine whether or not infection by *B. bassiana* affected sweepnet sampling efficiency, total sweep catches were adjusted against grasshopper density (sampling frames) by plot, date, and treatment; sweepnet collection has been used to determine grasshopper condition and activity (Johnson et al. 1986).

Results

Laboratory Assays. Conidial viability. Germination of conidia of GHA 92, GHA 94, and GHA STD exceeded 92% after 24 h (Fig. 1). However, the rates of germination differed ($F = 35.6$; df = 2, 21; $P \leq 0.01$) among conidial treatments. There was no difference ($P > 0.05$) in the prevalence of germination at 6 and 24 h, but a lower proportion ($P \leq 0.05$) of conidia of GHA 94 had germinated after 12 and 18 h.

Virulence assessments. The prevalence of mycosis was dissimilar ($F = 6.4$ and 7.1; df = 2, 14; $P \leq 0.01$) among conidial treatments after 8 and 14 days, respectively (Fig. 2). Averaged over dose, mycosis was less ($P \leq 0.05$) for GHA 94 than for GHA 92 and GHA STD. Correspondingly, the LD_{50} for GHA 94 was larger than that for the other two treatments (Table 1). Although there were no differences ($\chi^2 = 2.7$; df = 2; $P > 0.05$) in slopes of mortality 14 days after inoculation, slopes were dissimilar ($\chi^2 = 8.2$; df = 2; $P < 0.05$) among treatments 8 days after inoculation (Table 1; Fig. 2).

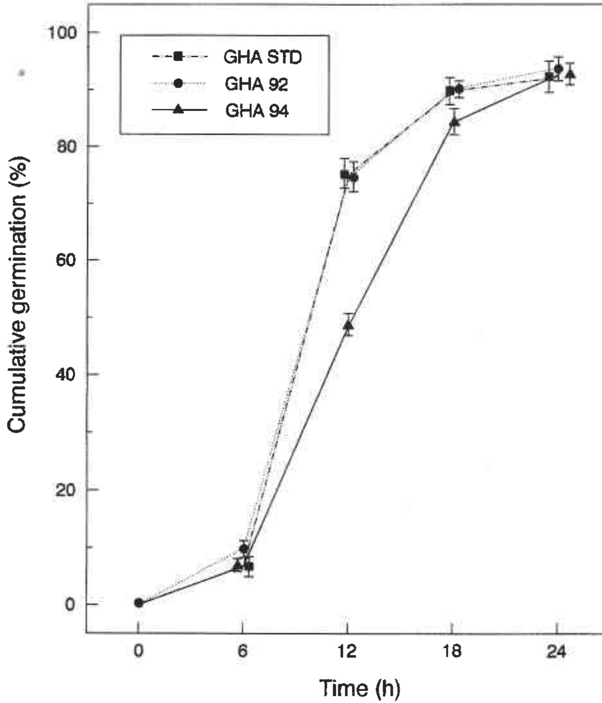

FIG. 1. Germination of conidia of *Beauveria bassiana* on potato dextrose agar at 25°C. Treatments consisted of *B. bassiana* (Mycotech strain GHA): conidia produced in 1992 (GHA 92); conidia produced in 1994 (GHA 94); and conidia of a standard batch (GHA STD) stored at −10°C.

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Field Trial. Application rates. There were no differences ($F = 0.2–4.7$; df = 2, 2; $P > 0.05$) among treatments in the deposition of spray droplets on water-sensitive papers (Table 2). The deposition of conidia on coverslips, leaves, and nymphs were also similar between the two conidial treatments. From coverslips, 4.1×10^4 (SE = 0.33×10^4) and 3.4×10^4 (SE = 0.29×10^4) cfu/cm^2 were recovered for GHA 92 and GHA 94, respectively. From grass leaves, 2.9×10^4 (SE = 0.28×10^4) and 2.4×10^4 (SE = 0.19×10^4) cfu/cm^2 were recovered, respectively. All nymphs ($n = 80$) processed after conidial application were

TABLE 1. Probit analysis of mycosis in nymphal *Melanoplus sanguinipes* inoculated with conidia of *Beauveria bassiana* and maintained in a controlled environment*

	Day 8			Day 14		
Treatment†	LD_{50}	*a*	*b* (SE)	LD_{50}	*a*	*b* (SE)
GHA STD	3.65 (3.49–3.80)‡	0.12	1.34 (0.16)	3.29 (3.00–3.49)‡	1.54	1.05 (0.15)
GHA 92	3.30 (2.90–3.56)	2.42§	0.78 (0.14)§	2.85 (2.22–3.18)	2.73	0.79 (0.15)
GHA 94	4.02 (3.86–4.17)	0.13	1.24 (0.15)	3.79 (3.61–3.96)	0.72§	1.13 (0.15)

*Results from two trials were combined. The regression equation is probit mycosis = $a + b$ ($\log_{10}$ dose).
†Conidial treatments consisted of *B. bassiana* (Mycotech strain GHA): conidia produced in 1992 (GHA 92); conidia produced in 1994 (GHA 94); and conidia of a standard batch (GHA STD) stored at −10°C.
‡Numbers in parentheses following LD_{50} value means represent 95% fiducial limits.
§Slopes or intercepts significantly different ($P \leq 0.05$) from those of other treatments.

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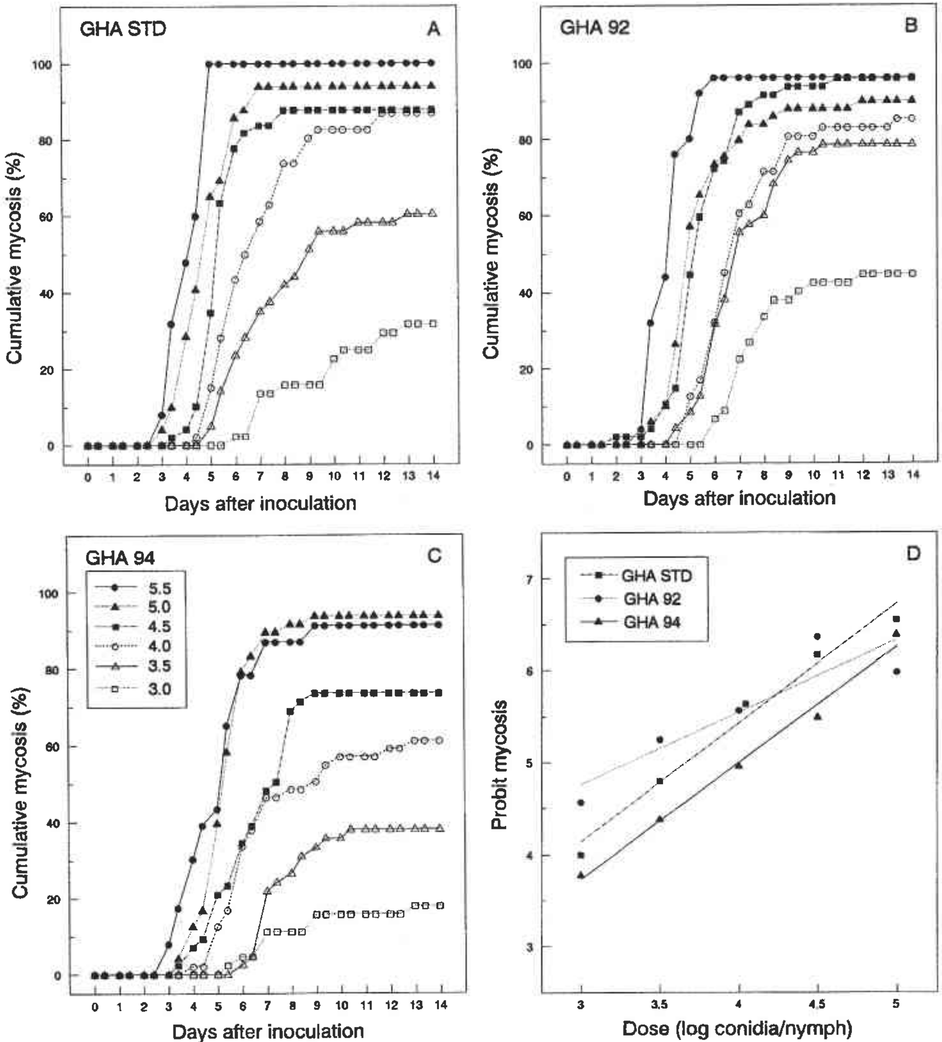

FIG. 2. Disease progress curves for grasshopper (*Melanoplus sanguinipes*) nymphs ingesting lettuce disks inoculated with conidia of *Beauveria bassiana* in oil containing either 0, 3.0, 3.5, 4.0, 4.5, 5.0, or 5.5 $\log_{10}$ conidia per lettuce disk: (A) GHA standard (GHA STD); (B) GHA 92; and (C) GHA 94. (D) Probit regressions of mycosis at 8 days for each treatment by dose.

TABLE 2. Deposition of spray droplets on water-sensitive cards placed on the soil surface within field plots sprayed with conidia of *Beauveria bassiana* or the carrier alone*

Treatment†	Droplet area (mm^2)	Droplet width (mm)	Coverage (%)	Number (per cm^2)
GHA 92	0.17 (0.028)	0.35 (0.013)	12.3 (3.6)	71.1 (7.1)
GHA 94	0.19 (0.011)	0.36 (0.031)	12.9 (3.4)	69.1 (21.1)
Carrier	0.10 (0.017)	0.27 (0.032)	9.9 (0.054)	112.1 (24.5)

* Droplets were analyzed in each of three randomly selected areas (80 mm^2) per card; the droplet data from the three areas were combined and mean droplet area, width, and density were determined. In addition, the ratio of total droplet area to total card area (% coverage) was calculated.

† Treatments consisted of conidia of *B. bassiana* produced in 1992 (GHA 92); conidia produced in 1994 (GHA 94); and a carrier control.

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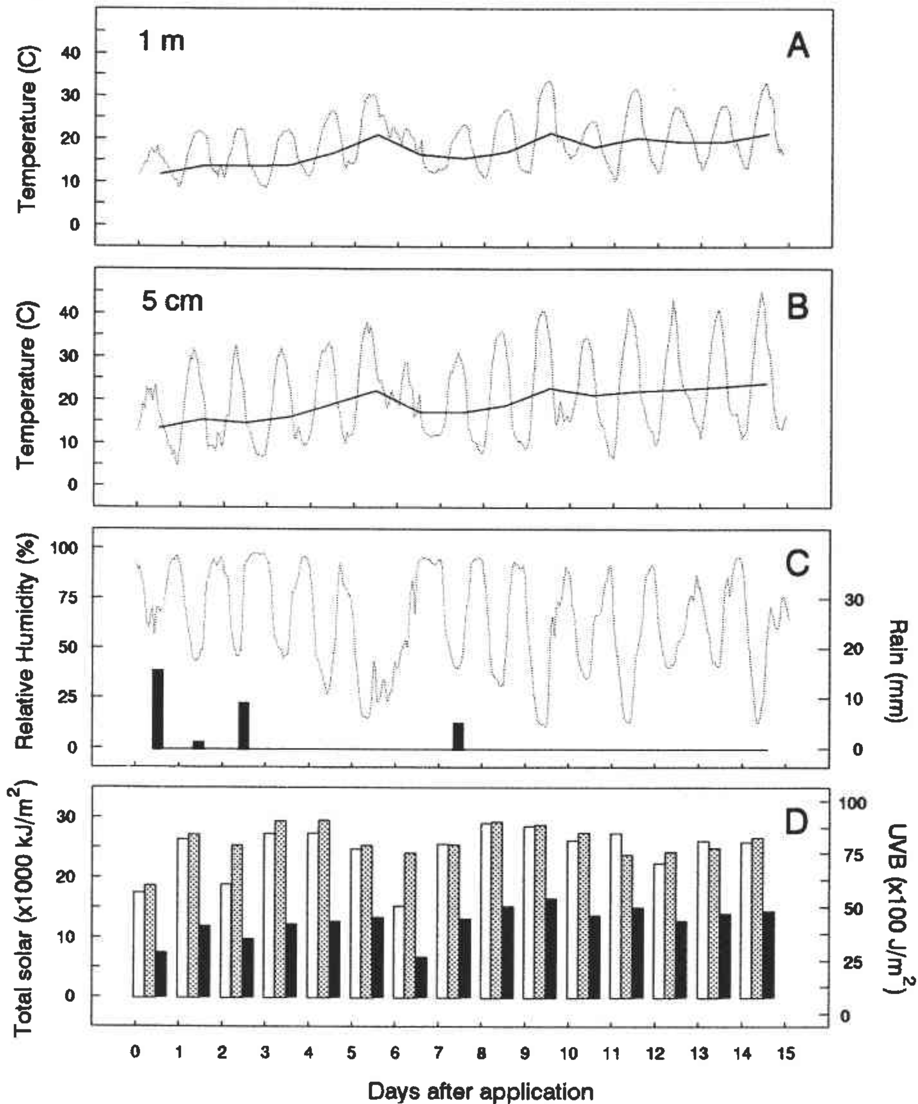

FIG. 3. Weather during the field experiment 12–27 July 1994. Hourly (dotted lines) and mean (solid lines) temperature at a height of 1 m and 5 cm, and relative humidity were recorded at Lethbridge. Daily precipitation (histograms) was recorded at Taber. Total daily solar radiation (300–2800 nm) was recorded at Lethbridge (open bars) and Vauxhall (stippled bars). Daily UV-B radiation (solid bars) at Lethbridge. Conidia of *Beauveria bassiana* were applied on the morning of 12 July.

infested with *B. bassiana*. Conidial populations on nymphs averaged 4.3×10^3 (SE = 0.39×10^3) and 3.5×10^3 (SE = 0.65×10^3) cfu/nymph for GHA 92 and GHA 94, respectively. Low levels of *B. bassiana* were recovered from carrier control nymphs (78.7 cfu/nymph), whereas *B. bassiana* was not isolated from coverslips or leaves from the carrier control treatment.

Weather conditions. In general, conditions were hot and sunny during the course of the experiment (Fig. 3). Four periods of precipitation occurred, with approximately 16 mm of rain falling within 24 h of the conidial application.

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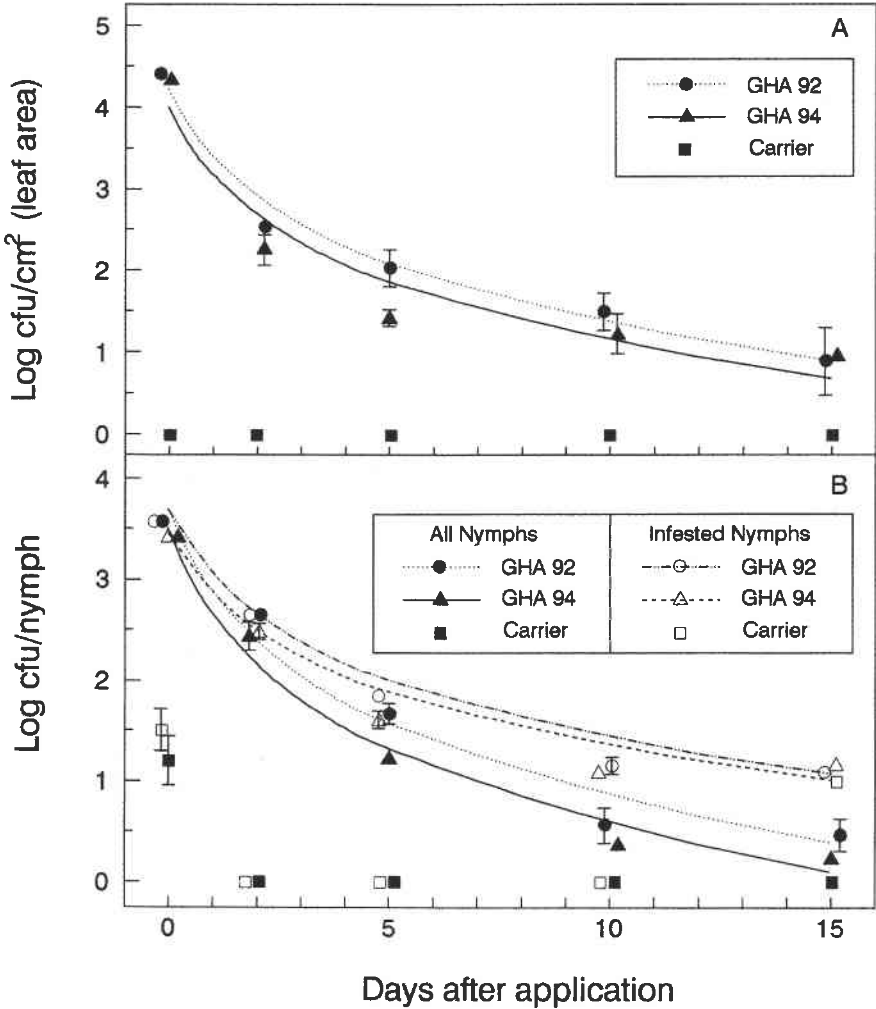

FIG. 4. Persistence of conidia of *Beauveria bassiana*: (A) from grass leaves [populations were quantified as $\log_{10}$ colony-forming units (cfu)/cm^2 of leaf area]; (B) from grasshopper nymphs (populations were quantified as log cfu per nymph). To account for variation in nymphal size, weights were standardized to 48 mg. Conidial populations were determined for total nymphs and for nymphs from which one or more cfu were recovered (classified as infested). Treatments consisted of GHA 92, GHA 94, and a carrier control. Vertical lines represent standard errors of means ($n = 2$). To avoid superimposition of standard error bars, means are slightly offset along the x-axis.

Conidial persistence. Conidial survival on leaves declined logarithmically over time (Fig. 4A). Slopes of conidial persistence were the same for GHA 92 (–2.8, SE = 0.26) and GHA 94 (–2.8, SE = 0.45). No conidia were recovered from carrier control leaves. Conidial survival on or in nymphs was similar (–2.8, SE = 0.22 and 0.25) to that observed on leaves (Fig. 4B). The prevalence of nymphal infestation also decreased with sample time to less than 30% by 15 days (Fig. 5A). When non-infested nymphs were removed, slopes of conidial survival decreased from –2.8 or –2.2 or less. Of the carrier control nymphs collected immediately after application, 80% (SE = 0.05) were infested with *B. bassiana* at low levels ($\bar{X} = 49.0$, SE = 29.7 cfu/nymph). With the exception of day 15 (1 of 40 nymphs), no viable conidia were recovered from nymphs at later sampling times.

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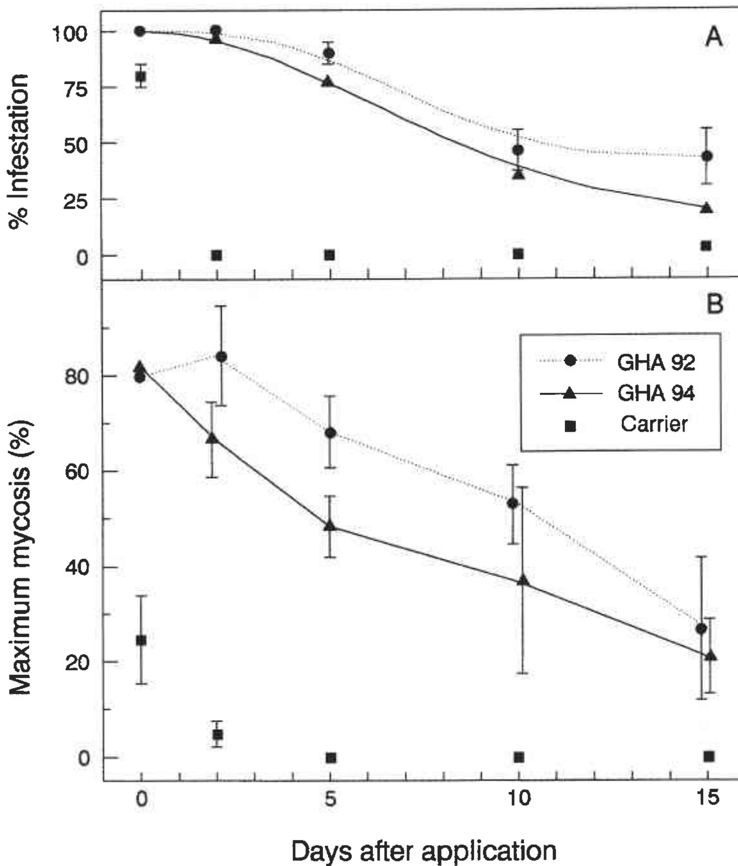

FIG. 5. (A) Infestation of grasshopper nymphs with *Beauveria bassiana* following field application. The percentage infestation was calculated from 40 nymphs collected per sample time. (B) Maximum mycosis in grasshoppers maintained in cages for 10 days per sample time. Treatments consisted of GHA 92, GHA 94, and a carrier control. Vertical lines represent standard errors of means ($n = 2$). To avoid superimposition of standard error bars, means are slightly offset along the x-axis.

Mycosis in field-collected grasshoppers. Over 2800 grasshoppers were collected and confined in greenhouse cages for 10 days. At individual collection dates, the timing of mycosis was similar ($F = 0.18–2.0$; df = 10.20, 2; $P \geq 0.05$) between GHA 94 and GHA 92 (Fig. 6). For both conidial treatments, the prevalence of mycosis decreased ($F = 14.8$; df = 4.8; $P \leq 0.001$) with sample time; beginning with the 5-day sample, less mycosis ($P \leq 0.05$) was observed (Fig. 5B). However, the onset of mycosis was always 3–4 days after placement of the nymphs in cages regardless of the collection time. There was no difference ($F = 0.33$; df = 2, 12; $F = 0.72$) between treatments in the prevalence of mortality not associated with *Beauveria*.

Grasshopper populations, species, and age composition. At the time of application, population densities were approximately 20 grasshoppers per square metre. None of the treatments affected ($F = 0.64$; df = 9, 12; $P = 0.74$) field populations of grasshoppers (Fig. 7). However, populations were consistently higher ($P < 0.05$) in the unsprayed plots. The predominant grasshoppers (comprising more than 10% of the 4333 individuals

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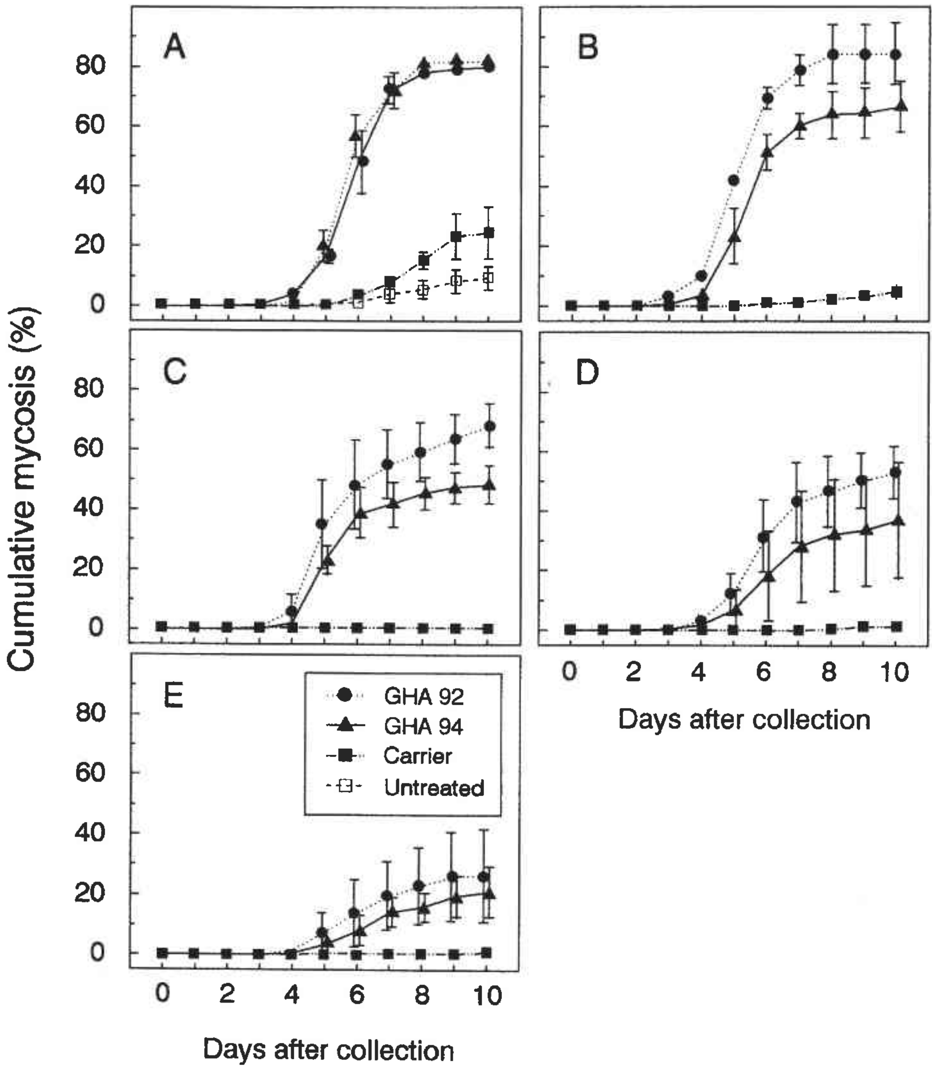

FIG. 6. Cumulative mycosis of caged grasshoppers collected from field plots at various times after the application of conidia of *Beauveria bassiana*. Grasshoppers for the GHA 92, GHA 94, a carrier control, and untreated control treatments were maintained in a greenhouse for 10 days post-application. Grasshoppers were collected: (A) immediately after the application of conidia; (B) 2 days after application; (C) 5 days after application; (D) 10 days after application; and (E) 15 days after application. Vertical lines represent standard errors of means ($n = 2$). To avoid superimposition of standard error bars, means are slightly offset along the x-axis.

collected) were *Ageneotettix deorum* (Scudder) (2–18%), *Melanoplus gladstoni* Scudder (10–28%), *M. infantilis* Scudder (14–36%), *M. packardii* Scudder (0–16%), *M. sanguinipes* (1–17%), and *Phoetaliotes nebrascensis* (Thomas) (24–47%). No remarkable shifts were observed in species composition in the treated or control plots over the four sampling dates. Less than 9% of the grasshoppers at the field site were adults at the time of application and most of these were small, early-season species (i.e. *M. infantilis*). By

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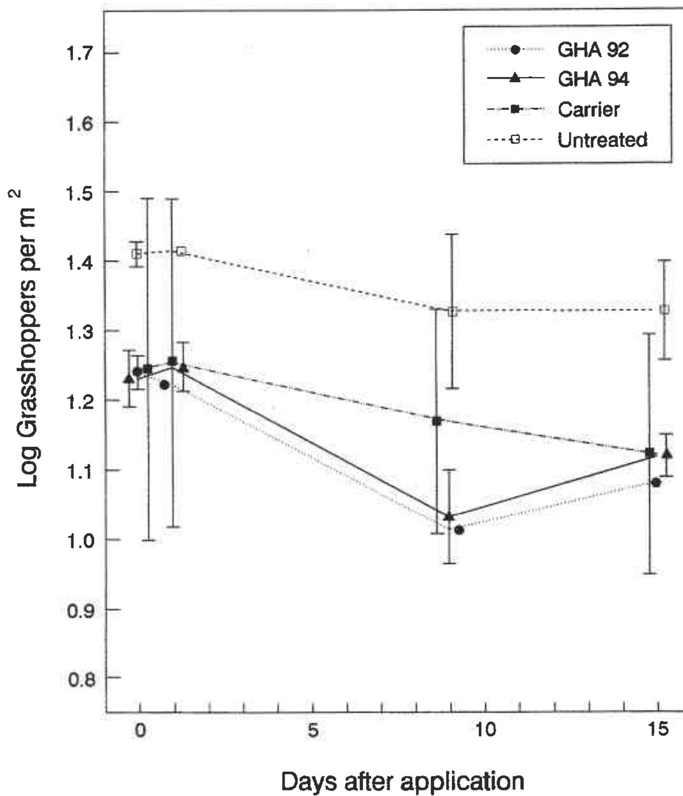

FIG. 7. Grasshopper densities in sampling frames ($\log_{10}$ grasshoppers per m^2) for the GHA 92, GHA 94, carrier control, and untreated control treatments taken on the day of application of conidia of *Beauveria bassiana*, and 1, 5, and 9 days after conidial application. Vertical lines represent standard errors of means ($n = 2$). To avoid superimposition of standard error bars, means are slightly offset along the x-axis.

15 days, 25.8% of the grasshoppers collected were adults. Cooler than average weather in the spring and summer of 1994 accounted for the low proportion of adults at the time of conidial application; accumulated degree-days (base 15°C) for the period from 1 May to 12 July were 153 compared with the decade average of 179 degree-days for the same period.

The 200 sweeps per plot yielded an average of 135 grasshoppers. At each of the sample times, there were no differences ($F = 0.06–4.4$; df = 3, 3; $P > 0.05$) among treatments in the catch per unit density (sampling frames). The ratio of grasshoppers caught in sweepnets to population densities ranged from 13.4 (SE = 4.9) to 25.8 (SE = 1.7) at the time of application. At the 1-, 9-, and 15-day sample times, ratios ranged from 21.3 (SE = 3.4) to 35.6 (SE = 0.87), 33.4 (SE = 0.26) to 51.6 (SE = 7.3), and 32.0 (SE = 0.10) to 46.1 (SE = 1.8), respectively.

Discussion

The poor field efficacy of entomopathogens has frequently been blamed, in many instances without corroborating evidence, on poor viability of propagules, reduced virulence, inadequate targeting of the host, and combinations thereof. We examined the activity of two production batches of conidia of *B. bassiana* (GHA 92 and GHA 94) in laboratory and field environments, to determine if previous differences in field efficacy could be

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explained by changes in the pathogen or by other aspects of the pathogen–grasshopper–environment interaction.

The ability of conidia of *B. bassiana* to germinate rapidly and synchronously is considered an important aspect of the infection process. Although conidia of GHA 94 germinated more slowly than did conidia of GHA 92, both were equally viable (>92%) after 24 h. Both conidial treatments were also highly virulent against nymphal *M. sanguinipes* in a controlled environment, with observed LD_{50} values of <2.9 and 3.8 log conidia per nymph for GHA 92 and GHA 94, respectively. Some evidence suggests that the rate of conidial germination may be important (Ouedraogo 1993) and this may explain why GHA 92 was slightly more virulent than GHA 94 against nymphal *M. sanguinipes* in our laboratory bioassay. In grasshoppers collected from field plots treated with GHA 92 and GHA 94 and subsequently held in cages in the greenhouse, we observed similar levels of mycosis for both treatments. Although non-significant, consistently less mycosis (excluding the day 0 sample) was observed in grasshoppers sprayed with GHA 94 than in those sprayed with GHA 92, and this may reflect reduced virulence. Nevertheless, high levels of mycosis were observed in caged grasshoppers collected from plots sprayed with GHA 94, and the previous dissimilarity in the field efficacy of GHA 92 and GHA 94 cannot be explained by either differential virulence or conidial viability alone.

Adequate host targeting is imperative for successful inundative control of insects with entomopathogenic fungi. We observed the uniform deposition of spray droplets and recovered relatively large populations (>10^4 conidia/cm^2) of both GHA 92 and GHA 94 from coverslips and leaves. Furthermore, all of the grasshopper nymphs we sampled following application were infested with *B. bassiana* (10^3 to 10^4 conidia per nymph). The homogenization method usually provides a conservative estimate of conidial populations because of the strong attachment of conidia to insect integuments (Boucias et al. 1988), to conidial aggregation, or both. However, some transmission of conidia of *B. bassiana* may have occurred between nymphs and from infested foliage during their capture in sweepnets, resulting in an overestimation of conidial populations. The wind direction at the time of conidial application would have precluded much wind contamination of control plots, yet we observed a high prevalence (80%) of carrier control nymphs infested with conidia, albeit at low levels (<80 cfu/nymph). These nymphs were inadvertently exposed to thatch from treated cages during their transport to the laboratory and likely became contaminated at this time; after the first collection time, cages were separated from each other by a plastic barrier and very few conidia (≤0.46 cfu/nymph) were subsequently recovered. In preliminary application tests using soluble dyes, excellent topical coverage of grasshoppers in sparse canopy grassland was observed (unpublished data). Therefore, it is highly probable that many of the conidia that we recovered were deposited on nymphs during the spray application. Despite the deposition of relatively large numbers of conidia on grasshoppers, we observed no reductions in field populations suggesting that parameters other than targeting and pathogen virulence limit disease development.

For mycosis to occur, conidia of *B. bassiana* must be deposited on grasshoppers in a suitable environment. After a cool and overcast application day, weather conditions were hot, dry, and sunny during the field experiment. Although we observed no evidence of disease in the field, substantial mycosis of grasshoppers treated with GHA 92 and GHA 94 was observed in greenhouse cages, a phenomenon observed by others (Johnson et al. 1992; unpublished data in Mason and Erlandson 1994). In the greenhouse cages, the prevalence of mycosis was observed to decrease with sample time, but the onset of disease was always 3–4 days after the placement of nymphs in cages regardless of when the grasshoppers were collected. Johnson and Goettel (1993) also observed a lag period of mycosis in caged grasshoppers, but because disease in cages corresponded to reductions in field populations of grasshoppers treated with *B. bassiana*, they speculated that the lag period was a result of

the differential collection of diseased and non-diseased grasshoppers; grasshoppers in an advanced stage of infection can become less active and, therefore, less likely to be collected in sweepnets (Johnson 1989). However, the ineffectiveness of *B. bassiana* against field populations of grasshoppers in our study indicates that the cage environment and not the differential collection of grasshoppers influenced disease development.

The influence of environment on mycosis of grasshoppers could be direct or indirect or both. The survival of conidia exposed to UV-B radiation on leaves in field environments is poor (Inglis et al. 1993, 1995) and conidial populations of GHA 92 and GHA 94 were reduced by more than 99% by 15 days. Conidial persistence on nymphs was similar to that on leaves suggesting that, as on leaves, UV-B radiation limits conidial survival on nymphs. This assumes that most of the conidia on nymphs remain exposed to sunlight. Although the homogenization technique does not discriminate between surface conidia and blastospores present in the hemolymph, comparisons of sweepnet efficacy relative to population densities (sampling frames) provided no evidence for the grasshoppers' activity being reduced by infection. This would suggest that conidia did not initiate infection and remain exposed to UV-B radiation on grasshoppers' integuments, that infection occurred but was not debilitating, or both. Furthermore, in the diffuse light conditions of the greenhouse, it is probable that conidia on nymphs would be exposed to much lower levels of UV-B radiation, prolonging their survival and thereby contributing to the development of disease.

Relative humidity was higher (≤40% on average) in the greenhouse than in the field. Although there may be some effect of relative humidity on infection of acridids by entomopathogenic fungi (Bateman et al. 1993), infection of grasshoppers by *B. bassiana* readily occurs at low humidities (Marcandier and Khachatourians 1987). Another possibility is that the efficacy of *B. bassiana* in the greenhouse was positively influenced by temperature. Nighttime temperatures in the greenhouse were slightly higher (approximately 4°C) than in the field but the upper cardinal temperature for *B. bassiana* (approximately 35°C) was surpassed for only short periods of time. However, behavioral thermoregulation by habitat selection and orientation to the sun allows grasshoppers to maintain their metabolic rates at temperatures near that which is optimal for development (approximately 42°C) (Hardman and Mukerji 1982; Carruthers et al. 1992), and body temperatures 18°C higher than ambient have been recorded for *M. sanguinipes* in natural habitats (Chappell and Whitman 1990; Kemp 1986). The elevation of insect body temperature by thermoregulation has been shown to reduce the effects of disease (Carruthers et al. 1992; Watson et al. 1993), and it is possible that the poor light conditions or the inability of grasshoppers to elevate their body temperatures sufficiently by habitat selection in the greenhouse cages, or both, contributed to disease development. Whether or not the inability of grasshoppers to thermoregulate in the greenhouse cages, possibly in conjunction with the enhanced survival of conidia of *B. bassiana*, contributed to enhanced mycosis remains to be determined.

A threshold of inoculum is required to cause disease and the quantities of viable conidia of *B. bassiana* that we recovered from grasshoppers at later sampling dates would not normally be sufficient to cause mycosis. For example, only 20–43% of collected nymphs were infested at low levels (13–15 cfu/nymph) at day 15, yet 21–26% mycosis was observed. Confinement of grasshoppers in cages may have predisposed them to infection because a variety of factors including crowding (Steinhaus 1958), nutrition (Donegan and Lighthart 1989), prior sublethal infection (Fargues et al. 1991; Ferron 1981), and agrochemical exposure (Ferron 1971) have been shown to predispose insects to infection by *B. bassiana*. Regardless of the reasons for the differential activity of *B. bassiana* between the field and greenhouse, it is clear from our study that mycosis in caged grasshoppers does not indicate field efficacy, and extreme care should be taken in interpreting results obtained from cage studies.

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Conclusion

The two conidial batches of *B. bassiana* that we tested were efficacious against laboratory-reared grasshoppers despite previous reports of differential field efficacy. Although conidia from both treatments were uniformly deposited onto grasshoppers, we did not observe significant reductions in field populations. However, high levels of mycosis were observed in grasshoppers collected from the field and maintained in greenhouse cages. The prevalence of mycosis in the caged grasshoppers decreased with sample time, but the onset of disease was always 3–4 days after collection, suggesting that environmental conditions and not the quantity and quality of inoculum were responsible for the poor efficacy of *B. bassiana* in the field. Furthermore, the different efficacies of this entomopathogen in these two environments emphasize that disease in caged grasshoppers should not be used as evidence of field efficacy. If *B. bassiana* is to be used effectively to manage insect pests, a better understanding of the conditions necessary to initiate epizootics is essential.

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