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Effects of Temperature and Thermoregulation on Mycosis by *Beauveria bassiana* in Grasshoppers

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

**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, Canada V5A 1S6*

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The influence of behavioral thermoregulation by grasshoppers (*Melanoplus sanguinipes*) on mycosis caused by *Beauveria bassiana* was investigated in controlled environments. The cardinal temperature for *B. bassiana* conidial germination and hyphal development was approximately 35°C. A low prevalence of mycosis (≤7%) was observed in inoculated nymphs exposed to a continuous temperature of 35 and 40°C, whereas continuous exposure to 30°C did not have a significant effect on disease development. Daily exposures to 35 and 40°C for 1 and 6 h, respectively, decreased mycosis in nymphs. In both environments, a strong correlation ($r \geq 0.95$) was observed between hyphal growth on potato dextrose agar and final mycosis. Although high temperatures delayed conidial germination, only conidia exposed to continuous 35 or to 40°C for more than 8 h exhibited reduced germination after 24 h. The effects of temperature on conidial germination were poorly correlated with disease, and when nymphs were exposed to 35°C for 24 h, less mycosis was observed only in grasshoppers exposed between 1 and 2 days postinoculation. The thoracic temperature of nymphs permitted to bask adjacent to a heat source ranged from 38 to 42°C. In nymphs basking for 1 h per day, 46% less mycosis was observed, decreasing to 98% less disease in nymphs allowed to bask for 6 h or greater per day. On a heat gradient, a higher prevalence of *B. bassiana*-infected nymphs selected hotter positions than noninfected nymphs, suggesting a "behavioral fever" response to infection. This study indicates that high temperature and thermoregulation can adversely affect *B. bassiana* mycosis of grasshoppers and may explain the poor efficacy of this entomopathogen observed in some field experiments.

KEY WORDS: ***Beauveria bassiana*; mycosis; grasshoppers; *Melanoplus sanguinipes*; temperature; thermoregulation; behavioral fever.**

INTRODUCTION

The entomopathogenic fungus, *Beauveria bassiana* (Balsamo) Vuillemin, is being investigated as an alternative to chemical insecticides for the control of grasshoppers and locusts. However, the application of *B. bassiana* against field populations of grasshoppers has yielded inconsistent results (Johnson *et al.*, 1992; Lobo Lima *et al.*, 1992; Johnson and Goettel, 1993; Inglis *et al.*, 1996a). In a recent trial, no reductions were observed in field populations of grasshoppers despite the uniform application of a virulent genotype of *B. bassiana*, yet substantial mycosis was observed in grasshoppers held in greenhouse cages (Inglis *et al.*, 1996a). Although mycosis in cages decreased with sample time, the onset of disease was always 3 to 4 days after placement of the grasshoppers in the cages, suggesting that environmental conditions and not targeting or virulence limited the efficacy of *B. bassiana* in the field.

Acridids elevate their body temperatures higher than ambient via habitat selection and/or orientation to solar radiation (Chappell and Whitman, 1990; Heinrich, 1993). Thermoregulation by grasshoppers has been shown to reduce disease in controlled environments (Boorstein and Ewald, 1987; Carruthers *et al.*, 1992) but the effect of grasshopper thermoregulation on *B. bassiana* mycosis has not been studied. The objectives of this study were to: (1) measure the effect of varying exposures to high temperatures on mycosis in grasshopper nymphs; (2) determine whether nymphs provided the opportunity would elevate their body temperatures sufficiently to inhibit disease development; and (3) ascertain if nymphs exhibit a "behavioral fever" response to *B. bassiana* infection.

MATERIALS AND METHODS

Preparation of Inoculum

Viabilities of dry conidia of *B. bassiana* (GHA isolate, supplied by Mycotech Corp., Butte, MT) were determined in distilled water on potato dextrose agar (PDA) amended with 0.005% Benlate (DuPont, Wilmington, DE), 0.04% Pen G, and 0.1% streptomycin (Inglis *et al.*,

[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. E-mail: inglisd@em.agr.ca.

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1996a). Within 12 h of use, conidia were suspended in sunflower oil (Safflo, Unico Inc., Concord, ON) using a micropestle, and concentrations of conidia were estimated using a hemocytometer and adjusted to 2.0×10^5 viable conidia/ml.

Inoculation of Grasshoppers

The migratory grasshopper, *Melanoplus sanguinipes* (Fabricius), an oligophagous grasshopper that is a cosmopolitan pest of cultivated crops and grasses in North America (Vickery and Kevan, 1985), was used in all experiments. Nymphs of a nondiapause strain were maintained on a diet of bran and wheat seedlings at 20 to 25°C for 15 to 20 days; a vertical heat gradient was produced in the cage by a 25-W incandescent light bulb. Grasshoppers were inoculated according to Inglis *et al.* (1996b). Briefly, 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 that did not consume the entire disk during this period were removed from the experiment.

Continuous Exposure

Following inoculation, a minimum of 10 to 12 nymphs were individually transferred to 240-ml clear plastic cups. Nymphs were placed at either 25, 30, 35, or 40°C for 10 days in controlled environment chambers (CEC) under a 16/8 h photoperiod provided by fluorescent lights. Conditions of ambient and within-cup temperature and relative humidity were recorded with a CR21 micrologger (Campbell Scientific, Logan, UT). Nymphs were fed fresh wheat leaves and frass was removed from the containers daily. Dead nymphs were collected twice daily and cadavers were placed in petri dishes containing moistened filter paper at 25°C. Nymphs that survived the 10-day duration of the experiment were killed by freezing and placed on moistened filter paper. With the exception of the 25°C treatment (five replicates), the experiment was replicated four times (342 total nymphs).

Fluctuating Exposures

Groups of 20 to 21 inoculated nymphs kept singly in 240-ml cups were exposed to 35 or 40°C for 0, 2, 4, 6, 8, 12, or 24 h per day for 10 days in the CEC; a 1 h per day exposure to 40°C was also tested. The remainder of the day was spent at 25°C. Although the 35 and 40°C exposure experiments were conducted separately, both experiments were replicated four times (561 and 643 nymphs per temperature, respectively).

Conidial germination and colony development were examined after exposure to 35 and 40°C (times as above). For germination assessments, conidia were spread on PDA amended with Benlate, and germination rates were measured after 6, 12, 24, and 48 h for four replicate cultures conducted at different times. For colony growth determinations, 0.1 µl of a conidial suspension was centrally placed onto unamended PDA, and diameters were measured at 24-h intervals for 10 days by taking the mean of two perpendicular measurements. The experiment consisted of four replicate cultures per treatment.

Short Duration Exposures

Following inoculation, 20 to 22 nymphs per treatment were individually placed in 240-ml cups. Nymphs were exposed to 35°C for 24 h, immediately after inoculation, and at 1-day intervals until Day 4. The remainder of the 10-day incubation period was spent at 25°C with control treatment consisting of inoculated nymphs not exposed to 35°C. The experiment was replicated three times (310 total nymphs).

Basking Nymphs

Nymphs inoculated with *B. bassiana* conidia were allowed to move vertically in a heat gradient provided by a 25-W incandescent light bulb (General Electric, Missassauga, ON) in $40 \times 40 \times 30$-cm aluminum cages (Fig. 1). Less than 0.3 W of ultraviolet-B radiation (280–320 nm) is emitted from incandescent bulbs, representing 0.04% of the radiation output (General Electric). The cages were equipped with a perforated metal floor to prevent contact with frass. The bulb was mounted on the back wall, 21 cm (bulb center) from the bottom of the cage. A plastic mesh tube (8-cm diameter $\times$ 28-cm high) was placed upright on one side of the bulb to permit the vertical movement of nymphs. At various positions in the cage, temperatures were recorded with a CR21X micrologger (Campbell Scientific)

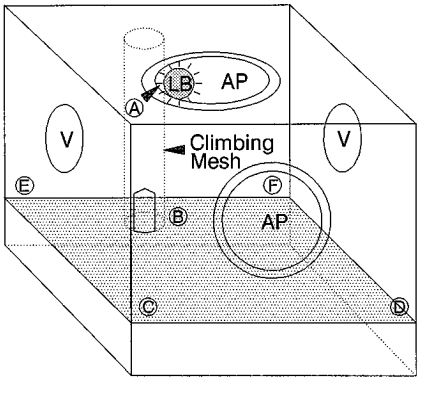

FIG. 1. To elevate their body temperature by basking, nymphs had to climb a plastic mesh (8-cm diameter $\times$ 28-cm high) toward a heat source (25-W incandescent light bulb; LB) in a aluminum cage ($40 \times 40 \times 30$ cm). The cage was equipped with a perforated metal floor and a clear Plexiglas top and front. Air flow was enhanced by mesh vents (V) and access ports (AP), and temperatures were monitored at points A to F.

equipped with eight wire thermistors (approximately 0.5 mm diameter).

Following inoculation, 47 to 50 arbitrarily selected nymphs per treatment were placed in each of eight cages kept in a CEC under a 16/8 h light/dark photoperiod provided by fluorescent bulbs. Nymphs were maintained on a diet of wheat seedlings (provided twice daily) and wheat bran for 10 days. Nymphs were exposed to the heat gradient generated by the light bulb for periods of 1, 2, 4, and 6 h per day; times of 12 and 24 h were also tested in two replicates but were not included in the analysis. The control treatment consisted of nymphs not exposed to a heat gradient. Cadavers were removed from the cages twice daily and placed on moistened filter paper in petri dishes. The experiment consisted of four to six replicates (1200 total nymphs).

To determine the temperature of basking nymphs, a copper constantan thermocouple (0.127 mm diameter) was inserted into the hemocoel (thorax between the right meso- and meta-sternites) of each of five third- and fourth-instar nymphs. These nymphs were positioned on the climbing mesh tube among thermoregulating nymphs (same height and orientation) and thoracic temperatures were output to a datalogger at 5-s intervals until they reached an asymptote. The thermocouple alone was also placed adjacent to nymphs on the mesh surface. In addition, the thoracic temperatures of nymphs were recorded at various positions on the floor of the cage.

Behavioral Fever

Treatments consisted of *B. bassiana*-inoculated and oil-treated (control) nymphs. For each treatment, approximately 25 to 30 arbitrarily selected nymphs were grouped in 21 × 28 × 15-cm Plexiglas containers equipped with a perforated metal floor and maintained at 25°C in the CEC under a 16/8 h photoperiod. Within 30 min of inoculation, and at 1-day intervals for 4 days, 20 nymphs per treatment were placed on a thermal gradient. With the exception of the Time 0 nymphs which had been starved for 12 h, nymphs were fed fresh wheat leaves approximately 12 h prior to placement on the gradient. The gradient consisted of an aluminum tray (92 × 16 × 8 cm) divided into two equal compartments along its length with a Plexiglas divider. One end of the tray was rested on a hot plate (Thermix, Model 310T, Fisher Scientific, Ottawa, ON), and eight surface temperature gradients (30 to 46°C) were marked in 2°C increments. To prevent nymphs from climbing the sides of the tray or onto the Plexiglas divider, cage walls were coated with a Teflon spray (Super Lube, Permatex Industrial, Newington, CT). At 30-min intervals for 4 h, the number of nymphs in each gradient division was recorded; nymphs in each gradient were combined across observation times ($n = 8$) for analysis. At the end of the observation period, nymphs were transferred back to the Plexiglas cages and maintained on a diet of wheat for 10 days. Subsequent mycosis in these nymphs by day was compared to inoculated and control nymphs maintained at a constant 25°C. The experiment was replicated four times (600 total nymphs), with the exception of the nymphs placed on the gradient within 30 min of inoculation (five replicates).

Statistical Analyses

All experiments were arranged as randomized complete block designs, and with the exception of colony growth, replicates were conducted in time. Nymphs on moistened filter paper that produced hyphal growth of *B. bassiana* were considered to have died from mycosis; those not producing hyphal growth were classed as "other mortality." There were no differences ($P > 0.05$) in other mortality between treatments, and unless indicated otherwise, mortality ranged from 0 to 18%. Residuals were plotted against predicted values to ensure homogeneity of variance, and where necessary an appropriate transformation was made prior to analysis. However, untransformed values for the means and standard errors of the mean (SE; in parentheses) are presented. Comparisons of disease progress and *B. bassiana* colony development between treatments were analyzed as split-plots in time (Gomez and Gomez, 1984). Since gradients were nonrandomized (behavioral fever experiment), treatment and replicate effects were also analyzed as a split-plot. For all split-plot analyses, a Box correction was used as a conservative test for the nonrandomized variables (x) and their interactions (Milliken and Johnson, 1984); the Box correction reduces the degrees of freedom for x, the x-treatment interactions, and the residual error(time) by $x - 1$. Subsequent to a significant F test, means were compared using the least-squares means function of SAS (SAS Institute Inc., 1988). Conidial germination, colony size, and mycosis at 10 days were compared at 35 and 40°C as a factorial experiment with replicates nested in temperature.

RESULTS

Continuous Exposure

Temperatures in the 240-ml cups were always similar (≤1°C) to ambient. In contrast, relative humidity in the cups was higher (≤36%) than ambient after the addition of wheat leaves each morning. The greatest rate of water loss from leaves occurred in cups maintained at the hotter temperatures. At all temperatures, humidity in the cups decreased as the leaves dried.

The rate of disease development ($F = 18.0$; df = 3,10; $P < 0.01$) and cumulative mycosis after 10 days ($F = 33.0$; df = 3,6; $P < 0.001$) differed between tem-

perature treatments (Fig. 2). Disease progress ($F = 1.2$; df = 1,6; $P > 0.05$) and the final prevalence of disease ($P = 0.27$) were similar between 25 (93.9%, SE = 3.6) and 30°C (77.5%, SE = 13.8). In contrast, considerably less ($P < 0.001$) mycosis (≤7.4%) was observed for the 35 and 40°C treatments after 10 days. In nymphs maintained at 40°C, a loss of cuticle pigmentation occurred, resulting in an "albino" appearance to the nymphs. Mortality not attributed to *B. bassiana* averaged 35.5% (SE = 16.7) for the 40°C treatment and less than 15.1% for the others.

Fluctuating Exposures

Increasing exposures to 35 and 40°C for varying periods each day influenced both the rate of disease development ($F = 34.8$–110.3; df = 6–7, 21–24; $P < 0.01$) and the prevalence of final mycosis ($F = 42.7$–191.4; df = 6–6, 18–21; $P < 0.001$) (Fig. 3). Less ($F = 30.7$; df = 6, 36; $P < 0.001$) mycosis was observed in inoculated nymphs exposed to 40°C than to 35°C, and minimum exposures of 1 and 6 h per day affected ($P < 0.04$) disease for the two temperature treatments, respectively.

Colony development was inhibited by daily exposures to 35 ($F = 76.9$; df = 6,28; $P < 0.001$) and 40°C ($F = 145.7$; df = 7,24; $P < 0.001$) (Fig. 4), but exposure to 40°C was more ($F = 22.2$; df = 6,42; $P < 0.001$) inhibitory. Hyphal growth was inhibited in cultures maintained at 35° for 4 h or longer per day and at 40°C for 1 h or more per day. Although a minimal increase (1.5 mm, SE = 0.24) was observed in colonies kept at a constant 35°C, conspicuous aerial tufts of white hyphae were observed. No growth was observed on cultures exposed to 40°C for ≥12 h per day. The effect of high temperatures on colony growth was highly correlated

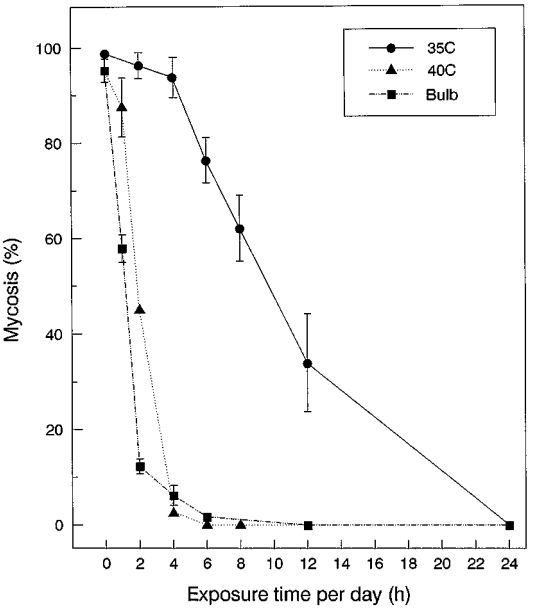

FIG. 3. Prevalence of mycosis (*Beauveria bassiana*) in *Melanoplus sanguinipes* nymphs (10 days) exposed to 35 or 40°C or to a vertical heat gradient (25-W incandescent light bulb) for varying times per day. Vertical lines represent standard errors of means ($n = 4$ to 6).

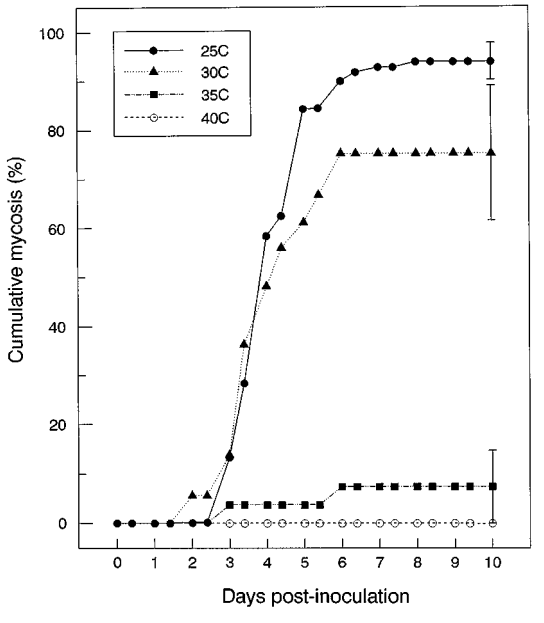

FIG. 2. Disease (*Beauveria bassiana*) progress curves for *Melanoplus sanguinipes* nymphs maintained at 25, 30, 35, and 40°C. Vertical lines represent standard errors of means for mycosis at 10 days ($n = 4$ to 5).

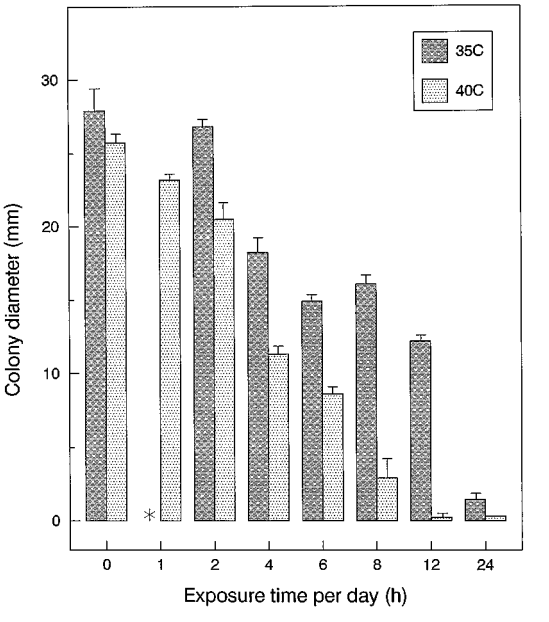

FIG. 4. Diameter (mm) of *Beauveria bassiana* colonies on potato dextrose agar (10 days) exposed to 35 or 40°C for various times per day. The asterisk indicates a missing datum point, and vertical lines represent standard errors of means ($n = 4$ to 5).

($r \geq 0.95$) with mycosis in grasshopper nymphs (Fig. 5). The best relationship was observed after logit-transformation of the mycosis data. The logit model used was ln $((p + 0.005)/1.005 - p)$, where p is the proportion of final mycosis.

Daily exposure to 35 ($F = 67.4$, df = 18,117; $P < 0.001$) and 40°C ($F = 50.4$; df = 21,62; $P < 0.001$) delayed conidial germination on PDA; 40°C was more ($F = 9.3$–52.8; df = 6,30–40; $P < 0.001$) inhibitory than 35°C (Figs. 6A and 6B). With the exception of conidia placed at a constant 35°C, there were no differences ($P > 0.05$) in conidial germination at 24 h between exposure treatments. In contrast, exposure to 40°C for ≥8 h per day reduced ($P \leq 0.05$) conidial germination at 24 h. Relative to colony growth, poorer relationships ($r = 0.47$ to 0.77) were observed between conidial germination after 24 h and mycosis in nymphs exposed to high temperatures.

Short Duration Exposure

Exposure of nymphs to 35°C for 24 h reduced the prevalence of final mycosis ($F = 3.9$; df = 4,8; $P = 0.048$) and slowed ($F = 3.7$; df = 4,10; $P < 0.05$) the rate of disease development. However, mycosis was only reduced ($P = 0.006$) in nymphs exposed to 35°C between 1 and 2 days postinoculation (46.7%, SE = 9.3). In nymphs exposed to 35°C at other times after inoculation, final mycosis ranged from 71.2% (SE = 15.8) to 81.9% (SE = 9.1). The prevalence of mycosis was 92.1% (SE = 4.1) in nymphs kept at a constant 25°C.

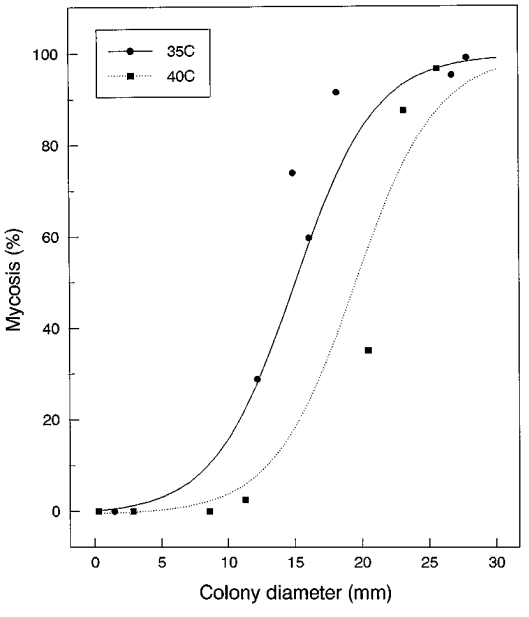

FIG. 5. Logistic relationship between *Beauveria bassiana* colony diameter (mm) on potato dextrose agar and mycosis of *Melanoplus sanguinipes* nymphs (%) exposed to 35 or 40°C for various durations each day.

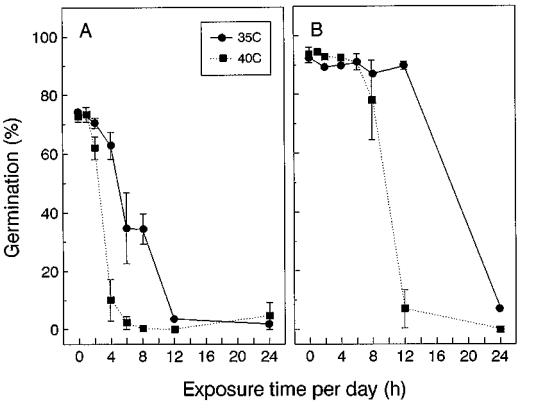

FIG. 6. Germination (%) of *Beauveria bassiana* conidia after various periods of exposure to 35 or 40°C per day: (A) germination at 12 h; (B) germination at 24 h. Vertical lines represent standard errors of means ($n = 4$ to 6).

Basking Nymphs

While the 25-W incandescent light bulb was on, temperatures within the cage ranged from 46 to 49°C on the climbing mesh at the closest proximity to the bulb (Fig. 1A), 26 to 27°C at the base of the mesh (Fig. 1B), and near ambient (25 to 26°C) at the other positions in the cage (Figs. 1C–1F). When the bulb was off, temperatures throughout the cage were similar to ambient (≈1°C). When switched on, nymphs quickly (ca. 1–5 min) climbed toward the heat source positioning themselves in a circle around the bulb; the internal thoracic temperature of both third- and fourth-instar nymphs ranged from 38 to 42°C. Basking affected both disease development ($F = 149.8$; df = 6,19; $P < 0.001$) and the prevalence of mycosis at 10 days ($F = 385.5$; df = 4, 12; $P < 0.001$) (Fig. 3). In nymphs allowed to bask for only 1 h per day, 46.0% less ($P < 0.001$) disease was observed relative to nonbasking nymphs.

Behavioral Fever

Nymphs starved for 12 h and placed on the gradient immediately after inoculation were more active than nymphs placed on the gradient at later times. By 3 days, a higher ($F = 14.1$; df = 1, 6; $P \leq 0.01$) frequency of nymphs inoculated with *B. bassiana* selected hotter positions on the heat gradient than nymphs treated with oil alone (Fig. 7). However, when the number of nymphs observed at the 40 to 42°C surface temperature gradient or greater were combined, a difference ($F = 6.9$; df = 4, 28; $P = 0.001$) between the control and *B. bassiana* treatments was detected ($P = 0.003$) in nymphs placed on the gradient by 2 days (Fig. 8).

Of the nymphs inoculated with *B. bassiana* but not placed on the gradient, 92.9% (SE = 3.1) died of mycosis after 10 days at 25°C. The prevalence of mycosis was also high in nymphs placed on the thermal gradient for

ca. 4.5 h; cumulative mycosis ranged from 71.5 (SE = 7.0) to 88.2% (SE = 8.3). Although the rate of disease development was similar ($F = 0.1$; df = 5, 45; $P > 0.05$) between treatments, the prevalence of final mycosis was less ($P \leq 0.018$) for nymphs placed on the thermal gradient 2 to 4 days postinoculation relative to those inoculated with *B. bassiana* but not placed on the gradient. Low levels of mycosis (≤7%) were observed in nymphs treated with oil alone.

DISCUSSION

Using behavioral mechanisms, acridids elevate their body temperatures by either directly or indirectly intercepting solar radiation (Chappell and Whitman, 1990; Heinrich, 1993). Although acridids are capable of raising their temperature substantially higher than ambient given the appropriate conditions, (Kemp, 1986; Carruthers *et al.*, 1992), the influence of thermoregulation by grasshoppers on the impact of entomopathogens has largely been ignored. We observed that mycosis in grasshoppers treated with *B. bassiana* was severely inhibited by continuous exposures to high temperatures. This finding is not surprising given the optimal (18 to 30°C) and upper thermal limit (approximately 35 to 38°C) for conidial germination and/or vegetative growth of *B. bassiana* (Roberts and Campbell, 1977; Fargues *et al.*, 1992). However, the impact of continuous high temperatures on mycosis is unrealistic since acridids elevate their body temperatures primarily during periods of sunlight.

Grasshoppers maximize the interception of solar radiation by habitat selection and/or by basking (Chappell and Whitman, 1990). To mimic basking, we exposed nymphs of the migratory grasshopper (*M. sanguinipes*) to a 25-W incandescent light bulb (heat source) placed adjacent to a climbing mesh. Nymphs quickly positioned themselves on the mesh in a ring around the bulb (Lactin and Johnson, 1996). The internal thoracic temperatures of these grasshoppers ranged from 38 to 42°C, which is consistent with reports of optima for development (Hilbert and Logan, 1983) and feeding (Lactin and Johnson, 1995) of *M. sanguinipes*. We observed that basking by grasshoppers severely inhibited mycosis caused by *B. bassiana*. In nymphs permitted to bask for only 1 h per day, 46% less mycosis was observed, and as the basking period increased, so did the inhibition of disease. Although there are costs associated with basking in nature (i.e., lost foraging time, increased parasitism, and increased predation), the benefits accrued may outweigh the costs. This study demonstrates that by elevating their body temperature by basking, grasshoppers can reduce the impact of disease caused by *B. bassiana*.

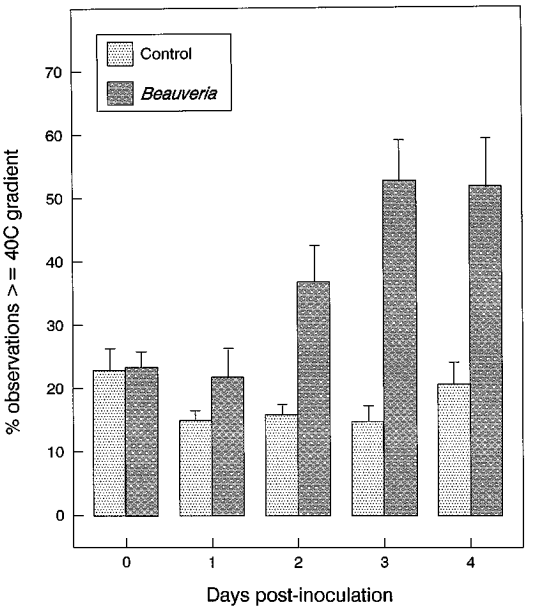

FIG. 8. Prevalence of *Beauveria bassiana*-inoculated and control *Melanoplus sanguinipes* nymphs (oil alone) observed on a thermal gradient at surface temperatures greater than or equal to 40°C (≥gradient 6). Vertical lines represent standard errors of means ($n = 4$ to 5).

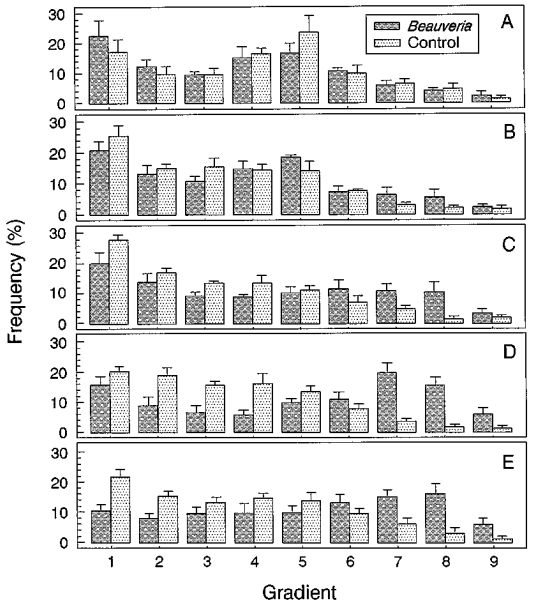

FIG. 7. Frequency distribution of *Beauveria bassiana*-inoculated and control *Melanoplus sanguinipes* nymphs (oil alone) on a thermal gradient. Gradients were marked at 2°C intervals (surface temperature) and ranged from 30 to 32°C (gradient 1) to greater than 46°C (gradient 9). Observations were made at 30-min intervals for 4 h, and nymphs per gradient per observation time were combined for final analysis. Positioning of nymphs on the gradient were analyzed at various times postinoculation (different nymphs were used): (A) immediately after inoculation; (B) 1 day after inoculation; (C) 2 days after inoculation; (D) 3 days after inoculation; and (E) 4 days after inoculation. Vertical lines represent standard errors of means ($n = 4$ to 5).

Grasshopper thermoregulation models indicate that in the prairie provinces of Canada and in the northern United States, grasshoppers would not necessarily be able to achieve a body temperature at or near their optimum (Kemp, 1986; Carruthers *et al.,* 1992; unpublished data). To determine the effects of suboptimal body temperatures on disease and to corroborate the results obtained from the basking experiment, we compared disease in nymphs exposed to 35 and 40°C for varying periods each day. Exposures to 35°C were substantially less detrimental than exposures to 40°C. However, an exposure time of 6 h or greater per day at 35°C inhibited mycosis, suggesting that even when conditions do not permit optimization of body temperature, thermoregulation by grasshoppers can influence mycosis. In nymphs exposed to 40°C, results were similar to those observed in the basking experiment. This would be expected given our supposition that acridids optimize their body temperature (ca. 40°C) by basking. In both the 35 and 40°C fluctuation experiments, conditions of relative humidity varied between treatments. This was attributed to differences in ambient relative humidity in the CEC and by the rate of water loss from wheat leaves (unpublished data). Although relative humidity represents a potential confounding factor in our experiments. Marcandier and Khachatourians (1987) showed no effect of relative humidity on the efficacy of *B. bassiana* against grasshoppers. We observed that disease development was similar between nymphs exposed to 40°C in cups and those allowed to bask (conditions of humidity increased ≤5% in the basking cages following feeding), further suggesting that temperature and not relative humidity was the primary factor affecting disease development.

In an attempt to elucidate the mechanism(s) by which temperature inhibits mycosis, we compared the effects of temperature on vegetative growth and of conidial germination on disease. Colony growth on PDA was more inhibited by exposures to 40 than 35°C, and strong correlations were observed between hyphal growth and mycosis at both temperatures. This suggests that the direct effects of temperature on growth of *B. bassiana* are important. We also observed that 30 to 47% of nymphs inoculated with *B. bassiana* and kept at 25°C for 4 days were cured by transfer to a high temperature (≥35°C) environment (unpublished data). It is uncertain whether the direct effects of temperature on *B. bassiana* alone are responsible for arresting disease development in nymphs at such an advanced stage of infection. The influence of high temperature on the physiology of the pathogen–host interaction has not been extensively studied, and it is seems highly probable that temperature also influences the immune response of insects.

The ability of conidia to germinate rapidly and synchronously is believed to be important in the infection process. The surface temperature of the cuticle in thermoregulating grasshoppers would be expected to be as high or higher than the temperature in the hemocoel. Although conidial germination was delayed by exposures to high temperatures, we found that the effect of temperature on germination was a poor predictor of mycosis. We also observed a reduction in mycosis only in grasshoppers placed at 35°C between 1 and 2 days postinoculation. Since the majority (≥92%) of *B. bassiana* conidia had germinated by 24 h at this temperature, this would seem to suggest that the early stages of pathogenesis (penetration and early internal proliferation) and not conidial germination are most affected by high temperatures. However, our germination assessments were made on PDA, and results obtained *in vitro* do not always correspond to those observed *in vivo.* Furthermore, conidia exposed on the surfaces of grasshoppers are rapidly deactivated by UV-B radiation in field environments (Inglis *et al.,* 1996a). Since our experiments were conducted under fluorescent lights, the consequences of delayed germination would be underestimated relative to a field environment.

Differential behavioral activity due to infection ("behavioral fever") has been reported in bacteria-infected cockroaches (Bronstein and Conner, 1984), in rickettsia-infected crickets (Louis *et al.,* 1986), in microsporidian-infected grasshoppers (Boorstein and Ewald, 1987), and recently in house flies infected with the entomopathogenic fungus, *Entomophthora muscae* (Watson *et al.,* 1993). We also observed a behavioral fever response to infection in *M. sanguinipes* nymphs infected with *B. bassiana.* The positioning of the nymphs on the gradient does not provide an accurate measure of internal body temperature since nymphs can affect their body temperature by changing postures. Nevertheless our observations of infected and healthy nymphs were conducted simultaneously, and as infection progressed, a higher frequency of *B. bassiana*-infected *M. sanguinipes* nymphs selected hotter positions on a heat gradient than noninfected nymphs. A number of factors other than infection by entomopathogens affect acridid behavior on thermal gradients. Chapman (1955) noted that starved nymphs (21–26 h) were more active than recently fed nymphs. This is consistent with our observations of increased activity in *M. sanguinipes* nymphs placed on the gradient immediately after inoculation (Time 0); these nymphs had been starved for ca. 12 h to facilitate ingestion of the lettuce bait. Subsequent nymphs (Times 1 to 4 days) were considerably less active on the thermal gradient than the Time 0 nymphs. However, our frequency distributions were, in general, wider than that reported by Chapman (1955) for locust nymphs (*Schistocerca gregaria*). Similar to Chapman (1955) we observed a disproportionate number of nymphs that became "trapped" at the cool end of the

gradient. Whether these nymphs became lost or were attempting to maintain a lower body temperature is uncertain (Heinrich, 1993). Our evidence of grasshopper behavior on a thermal gradient suggests that nymphs infected with *B. bassiana* will differentially elevate their body temperature in response to the infection. Most reports of behavioral fever in insects have been demonstrated on a thermal gradient, yet Carruthers *et al.* (1992) showed no difference in body temperatures of infected (*E. grylli*) or healthy nymphs while basking. The validity of our observations of a behavioral fever in *M. sanguinipes* nymphs infected with *B. bassiana* should be confirmed in basking experiments.

This study demonstrates that high temperature and thermoregulation by grasshoppers can inhibit/or prevent disease caused by *B. bassiana* and the ability of grasshoppers to elevate their body temperature may explain the variable efficacy of this entomopathogen in field environments. The ability of acridids to elevate their body temperatures is consistent with observations of successful control of grasshoppers with *B. bassiana* during cool overcast periods (Johnson and Goettel, 1993) and unsuccessful control during hot sunny periods (Inglis *et al.*, 1996a). Furthermore, the inability of acridids to thermoregulate in greenhouse cages may explain their increased susceptibility to *B. bassiana* in this environment (Inglis *et al.*, 1996a). It is also possible that high temperature affects the efficacy of *Metarhizium flavoviride* against acridids (Lomer, 1994). Although we have demonstrated that high temperatures and thermoregulation by grasshoppers can suppress mycosis caused by *B. bassiana* in controlled settings, confirmation of this phenomenon in a field environment is necessary.

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