



Temperature-sensitive *Beauveria bassiana* mycosis in the Mormon cricket, *Anabrus simplex*
by Jonathan Hunter Turnbow

A thesis submitted in partial fulfillment of the requirements for the degree Of Master of Science in
Entomology

Montana State University

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Abstract:

The GHA strain of the fungus *Beauveria bassiana* (Balsamo) Vuillemin was thermally constrained when used as a biocontrol agent on the Mormon cricket, *Anabrus simplex* Haldeman. In field cages, Mormon crickets thermoregulating normally in full sunlight had significantly lower fungal mycosis than those restricted to lower temperatures in shaded cages. Behavioral observations and body temperature measurements of field-caged and natural populations showed that the Mormon cricket's normal thermoregulatory range was higher than the level detrimental to *B. bassiana* growth. On a laboratory thermal gradient, Mormon crickets exhibited a small (0.2°C) but statistically significant behavioral fever after inoculation with the fungus. However, it is doubtful that Mormon crickets thermoregulating in the field could accurately express such a small increase in body temperature.

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MONTANA STATE UNIVERSITY-BOZEMAN
Bozeman, Montana

January 1998

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This thesis has been read by each member of the thesis committee and has been found to be satisfactory regarding content, English usage, format, citations, bibliographic style, and consistency, and is ready for submission to the College of Graduate Studies.

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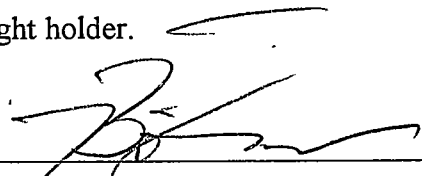
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ACKNOWLEDGEMENTS

First, I thank my major professor, Kevin O'Neill, for his guidance during my Master's program. His assistance and support allowed me to grow as a scientist, student, and instructor.

I am also grateful to my committee members, Robert Nowierski, Stefan Jaronski, and Tim McDermott, for their sacrifices in time and energy in improving my thesis and graduate experience. Dr. Nowierski and Brian Fitzgerald also were kind enough to allow me use of their thermal gradient.

Many of the personnel in the now-defunct USDA Rangeland Insect Lab were a great help to me, including: William Kemp, Douglas Streett, Elaine Oma, Jerry Mussnug, Ann de Meij, and Bill Johnson.

Steve Cherry, Robert Boik, Sharon Cebula, and Matt Bodoh all patiently assisted me with the PROC MIXED analysis.

Earl Lukkes and Allen Pomeroy with the Big Horn County, WY Extension Service helped me locate and collect Mormon crickets.

Ruth O'Neill assisted with the tedious job of watching caged Mormon crickets for hours, and Sam and Tom O'Neill were excellent cricket catchers.

Deanna Larson, Marni Rolston, and Cathy Seibert assisted with the construction of field cages. David Weaver helped me with the process of melding statistical output with biological reality.

Funding provided by a USDA-NRI Seed Grant and the Montana Agricultural Experiment Station.

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ABSTRACT

The GHA strain of the fungus *Beauveria bassiana* (Balsamo) Vuillemin was thermally constrained when used as a biocontrol agent on the Mormon cricket, *Anabrus simplex* Haldeman. In field cages, Mormon crickets thermoregulating normally in full sunlight had significantly lower fungal mycosis than those restricted to lower temperatures in shaded cages. Behavioral observations and body temperature measurements of field-caged and natural populations showed that the Mormon cricket's normal thermoregulatory range was higher than the level detrimental to *B. bassiana* growth. On a laboratory thermal gradient, Mormon crickets exhibited a small (0.2°C) but statistically significant behavioral fever after inoculation with the fungus. However, it is doubtful that Mormon crickets thermoregulating in the field could accurately express such a small increase in body temperature.

INTRODUCTION

Many ectotherms, including members of every major insect order, maintain some degree of control over their body temperatures, an operation known as thermoregulation. Behavioral changes in posture, orientation, and microhabitat selection allow some insects to maintain their body temperatures within a narrow range for several hours per day (May 1979, Casey 1981). The benefits of thermoregulation include: avoidance of hot and cold extremes, extension of habitats that can be occupied, optimization of growth rates, maximization of feeding, digestion, and defecation rates, and increased time for locomotion, feeding, and reproduction (Heinrich 1981, O'Neill 1994, O'Neill et al. 1994, Harrison and Fewell 1995).

Another benefit, only recently realized, is that many ectotherms thermoregulate to combat disease. Organisms that respond to disease by using behavior to increase their body temperature above the normal preferred temperature of uninoculated animals are exhibiting a behavioral fever (Horton and Moore 1993). Like endothermic fevers, behavioral fevers are part of the immune response of the organism; they aid in resisting and recovering from disease and infection. However, ectotherms rely upon their behavior and heat sources in the environment to produce a fever rather than the physiological mechanisms used by endotherms.

Behavioral fevers are well known in ectothermic vertebrates and arthropods including lobsters, scorpions, horseshoe crabs (Cabanac 1989), and reptiles (Kluger 1979). Insect behavioral fever was first reported in cockroaches by Bronstein and Conner (1984). Following that, researchers reported behavioral fever in beetles (McClain et al. 1988), crickets (Louis et al. 1986), grasshoppers (Boorstein and Ewald 1987), and houseflies

(Watson et al. 1993). The three latter studies also investigated the effects of fever on host disease resistance. All three found that behavioral fever reduced insect mortality or infection due to disease.

These observations raised concerns about the effectiveness of *Beauveria bassiana*, a fungal pathogen recently registered with the EPA by the Mycotech Corporation of Butte, MT, as a biocontrol agent for several species of Orthoptera. Target species that, at least sometimes, have body temperatures higher than the pathogen's upper thermal limit for growth (35°C [Inglis et al. 1996]) could reduce pathogenicity of *B. bassiana*.

Beauveria bassiana used in field plots to control nymphs of the migratory grasshopper, *Melanoplus sanguinipes*, yielded results contradictory to its proven efficacy under controlled laboratory conditions (Inglis et al. 1997a, 1997b). The disparity was due to high temperatures and grasshopper thermoregulation in the field. Inglis et al. (1996) also showed that *M. sanguinipes* nymphs inoculated with *B. bassiana* had a behavioral fever on a thermal gradient. The body temperature (T_b) of inoculated nymphs ranged from 38 - 40°C.

Other entomopathogenic fungi show a similar response to temperature. *Entomophaga grylli* has an *in vitro* upper thermal limit for survival and development near 35°C. Inoculated grasshoppers allowed to bask in simulated sunlight raised their T_b to 40°C and almost completely eliminated *E. grylli* mycosis. Grasshoppers held in diffuse light at 30°C had high rates of fungal mycosis (Carruthers et al. 1992). House flies inoculated with the related fungus, *Entomophthora muscae*, chose temperatures higher than controls in a thermal gradient. The flies in the thermal gradient also had a lower prevalence of mycosis than those inoculated and held outside the gradient at lower temperatures. The *in vivo* upper thermal limit for *E. muscae* growth was 32-35°C (Watson et al. 1993).

The objectives of my study were to: 1) examine Mormon crickets for a behavioral fever response when inoculated with *B. bassiana*, 2) determine whether the preferred

temperature ranges (T_{set} 's) found in #1 were possible under field conditions, 3) determine if given the opportunity would Mormon crickets thermoregulate at temperatures that inhibit fungal mycosis; and 4) examine the behavioral mechanisms used by Mormon crickets to regulate T_b in natural and controlled conditions.

Thermoregulation

Thermoregulation is the maintenance of a relatively stable T_b despite fluctuations in environmental conditions. Endotherms such as mammals and birds regulate T_b primarily by physiological mechanisms, but an ectothermic insect's primary mechanism for altering T_b is behavior. Most mammals and birds are regarded as endotherms because they use metabolic heat to maintain a relatively constant T_b . However, because most insects require external heat sources to thermoregulate they are termed ectotherms (Heinrich 1993).

Insects mimic thermoregulation in certain environmental situations without showing any responses adapted to that end. For example, a tree trunk is more sunlit at low than at high ambient temperatures (T_a). An insect that moved randomly about on the trunk would have a stable T_b throughout the day, but would not have to thermoregulate to do so (May 1979). Even a can of beer maintained a " T_b " different than T_a under the right environmental conditions (Heath 1964). Behavioral and physiological adaptations for stabilizing T_b indicate thermoregulation is occurring.

Behavioral thermoregulation is accomplished by manipulation of the four avenues by which insects exchange heat with their environment: radiation, convection, conduction, and evaporation. Most heat gain in insects comes from the absorption of solar radiation, and most heat loss from convective exchange with the air. The importance of conductance in heat exchange varies with the surface area of the insect contacting the substrate, and may be used for heating or cooling. Evaporative heat loss is usually

unimportant for insect thermoregulation due to inadequate reserves of body water (Casey 1981, Chappell and Whitman 1990) and would be especially difficult for Mormon crickets because of the arid climes they inhabit.

Physiological thermoregulation in insects is a result of: 1) endothermic heat production by large muscles, usually the flight muscles, and 2) mechanisms that distribute heat within the body (Heinrich 1993). Since Mormon crickets are wingless as adults, I will not review internal heating here. Instead, I will elaborate on the behavioral mechanisms insects, specifically grasshoppers, use to thermoregulate.

An insect uses postural and orientational changes and microhabitat selection to alter its heat exchange with the environment. By changing the interactions between their bodies and the radiative, conductive, and convective properties of their microhabitat, insects control their body temperature. The most common adjustments to posture and orientation are made in relation to the sun. Insects regulate the absorption of solar radiation by increasing or decreasing the surface area of their body exposed to the sun. Orientation so that the sun's rays strike perpendicular to the lateral surface causes a high rate of heat gain. Heat loss by convection can be increased by exposing more surface area to the wind and by orienting the long axis of the body perpendicular to it. Changes in the amount of surface area contacting the substrate affect rates of heat gain or loss by conductance (May 1979, Chappell and Whitman 1990).

Grasshoppers frequently exhibit postures correlated with T_b and T_a . *Psoloessa delicatula* exhibited 6 distinct postures (Anderson et al. 1979). In the early morning, the grasshopper basked with its long axis perpendicular to the sun's rays, and lowered its body to the ground into the crouched position. As T_a increased, basking continued, but the grasshopper raised its body the ground into the normal position. Grasshoppers used the stiling posture, body elevated by extension of the legs, at high soil surface temperatures (T_s). The posture reduced heat gain due to conductance and increased cooling due to

convection by raising the cricket's body above the layer of hot, stagnant air near the ground's surface. The dropped posture, abdomen lowered below wings without touching the ground, and the straddle posture, both hind legs spread away from the body, increased surface area for heat loss due to convection. The final posture observed was a combination of the dropped and straddle postures.

The lubber grasshopper, *Taeniopoda eques*, exhibited a similar set of postures (Whitman 1987). The flanking posture exposed the maximum body surface area to solar radiation. While the long axis was oriented perpendicular to the sun's rays, the grasshopper rotated its body to expose its broad lateral area to the sun. The hind leg on the sunny side was lowered to avoid shading the abdomen, and the hind leg on the shady side was elevated, increasing surface area exposed to the sun. Dropping the abdomen below the wings increased the abdominal surface area exposed to the sun. *Taeniopoda eques* pressed its ventral surface to the ground, the crouching posture, on cool, shady days when T_a was lower than soil temperature. The grasshoppers stilted on hot soil or roads. The lubber also frequently perched on the shady side of plant stems and kept the stem directly between its body and the sun.

A grasshopper from the Gobi desert, *Damalacantha vacca sinica*, crouched and flanked to raise its T_b above T_a (Toms 1991). In addition to the stilting posture, *D. vacca* reduced heat gain by using its pronotum as an umbrella. The body was oriented parallel to the sun's rays with the head and pronotum directed toward the sun. The large pronotal shield shaded the anterior portion of the abdomen.

The most frequent and effective method of behavioral thermoregulation in insects is the exploitation of thermally heterogeneous areas. Simply moving between sun and shade is adequate to maintain a constant T_b (Casey 1981). The small size of insects allows them to take advantage of the thermally diverse microhabitats available within small areas (Chappell and Whitman 1990).

Behavioral Fever

Behavioral fever occurs when an animal uses behavioral means to elevate its body temperature when diseased. By elevating T_b , an ectotherm may be able to eliminate or moderate pathogenesis due to the disease. Insects develop a fever with the same set of mechanisms used in thermoregulation, but elevate T_b above their normal T_{set} . Watanabe (1987) states, "Temperature is the most important external physical factor for both insect susceptibility and multiplication of microbial pathogens in the host."

Behavioral fever has been reported in ectothermic vertebrates such as fish, reptiles, and amphibians since the 1970's (Kluger 1979). In these groups, and in mammals, survival after infection increased to a point as the magnitude of the fever increased. Although invertebrate behavioral fever has only recently become an active area of study, the rearing of insects at a high constant temperature in order to eliminate disease has a long history. In 1946, infections in *Drosophila* due to a virus disappeared when larvae were reared at 30°C. Heat therapy at 35°C reduced a viral infection in the silkworm, *Bombyx mori*, in 1963 (Heinrich 1993).

The earliest report of a true invertebrate behavioral fever, rather than a no-choice rearing regimen, occurred in a crayfish in 1977. A bacteria-infected crayfish showed a fever of 2°C over baseline when placed in a thermal gradient (Casterlin and Reynolds 1977). There was no attempt to determine if the fever had any therapeutic effects. Subsequent research showed behavioral fevers in lobsters, horseshoe crabs, scorpions (Heinrich 1993), and leeches (Cabanac 1989).

Among insects, behavioral fever was first demonstrated for the Madagascar cockroach, *Gromphadorhina portentosa* (Bronstein and Conner 1984). When injected with a suspension of *E. coli*, the cockroaches showed a significant increase of 3.6°C in set-point temperature on a thermal gradient compared to control roaches.

Injection of *Rickettsiella grylli* into the cricket *Gryllus bimaculatus* resulted in a behavioral fever of 6.4°C on a thermal gradient (Louis et al. 1986). Ninety percent of the crickets at the febrile temperatures survived, while only 50% of the crickets held at a temperature 5°C lower survived. Incidence of rickettsial infection was significantly reduced in animals at febrile temperatures.

Melanoplus sanguinipes exhibited a fever of 6.3°C in a thermal gradient when infected with *Nosema acridophagus* (Boorstein and Ewald 1987). Infected grasshoppers held at the elevated temperature showed reduced mortality compared to infected grasshoppers at the nonfebrile temperature. The cost of a behavioral fever in *M. sanguinipes* was found to be a decreased growth rate in uninoculated grasshoppers held at febrile temperatures compared to uninoculated grasshoppers held at nonfebrile temperatures.

In addition to behavioral fever, insects may show other altered behaviors when parasitized or diseased. They may move to concealed locations, exposed locations, higher elevations, they may increase or decrease activity, or change the time of day for activity for reasons other than thermoregulation (Horton and Moore 1993). Depending on the specific circumstances, these behaviors can benefit host or parasite. If parasites are able to modify their host's behavior they could increase their survival and dissemination. A host organism's response could increase their own or kin survival. Determining the beneficiary of the behaviors becomes necessary. An elevated body temperature, if it increases the host's chance of reproduction, is an adaptation beneficial to the host. The occurrence of fever, behavioral and endothermic, across phyla and classes is evidence of its benefit to the host.

The Mormon Cricket

The Mormon cricket, *Anabrus simplex* Haldeman, is a shield-backed katydid (Family: Tettigoniidae) whose biology is well-suited to studying thermoregulation. Mormon

crickets are flightless and do not generate heat endothermically in flight or during a pre-flight warmup as some insects do. Mormon crickets normally inhabit arid rangelands and do not have the water reserves necessary to use evaporation to lose heat. Without endothermic heat production and evaporative cooling, Mormon crickets are limited to thermoregulating primarily by manipulating the amount of solar radiation absorbed, and the amount of heat exchanged convectively. These avenues of heat exchange are controlled by posture and microhabitat selection, both easily observable in such a large, slow moving insect.

The large size (2 - 4 g) of Mormon cricket adults also gives them considerable thermal inertia. Their high thermal inertia leads to more reliable estimates of T_b because the crickets are less likely to lose or gain significant amounts of heat between time of capture and T_b measurement.

Historically, Mormon crickets have been an agricultural pest. When conditions of cricket density and environment are right, Mormon cricket nymphs and adults coalesce into bands and can migrate 0.8 - 1.6 km per day. A single band can cover several hundred acres (Wakeland 1959). If a migrating band crosses agricultural lands serious damage can result from the omnivorous feeding by a large number of individuals.

One recent large Mormon cricket outbreak occurred in northeastern Utah and northwestern Colorado near Dinosaur National Monument beginning in 1985 (MacVean 1987). The aerial spraying of carbaryl was halted after one year because of concern for endangered species. Mormon cricket control primarily centered on breeding areas to prevent migration onto agricultural land. Therefore, pesticides were sprayed widely across thousands of hectares of rangeland onto what were frequently environmentally sensitive areas. A species-specific, nonchemical control method, such as an entomopathogenic fungus, would be better suited for Mormon cricket control when nontarget impacts are a concern.

Beauveria bassiana

Beauveria bassiana (Balsamo) Vuillemin, a member of the Fungi Imperfecti (Deuteromycetes), was the first microorganism recognized as an agent of disease. Bassi revealed in 1835 that *B. bassiana* caused white muscardine disease in the silkworm, *B. mori* (Carruthers and Soper 1987). Early reports of *B. bassiana* infection occurring in Orthoptera are from Schaefer in 1936 and MacLeod in 1954 (Marcandier and Khachatourians 1987). In 1995, the Mycotech Corporation (Butte, MT) was licensed by the EPA to use *B. bassiana* strain GHA as a biocontrol agent against Mormon crickets and other insects.

Beauveria bassiana does not infect Orthopterans through the gut but rather through the integument. After a conidium attaches to and germinates on the insect's integument, it produces hyphae that penetrate the body wall using both enzymatic and physical processes (Tanada and Kaya 1993). Once hyphae reach the hemocoel they produce budding yeastlike bodies. Death of the host results, not from attack of specific tissues, but by nutrient depletion and/or the presence of mycotoxins. After approximately 8 days conidiophores emerge from the cadaver and produce conidia (Streett and McGuire 1990).

Infection by *B. bassiana* induced a humoral response in the grasshopper *M. sanguinipes* (Gillespie and Khachatourians 1992). In the hemolymph, the activity of phenoloxidase, an enzyme leading to the formation of melanin, increased within 10-60 min of injection of viable *B. bassiana*, and showed a secondary increase 24 hr after injection. Wounding of the cuticle or injection of heat-killed *B. bassiana* resulted in the primary but not secondary phenoloxidase activity increases. Protein concentrations showed similar rapid increases for viable and non-viable injections, but only viable *B. bassiana* elicited a long-term increase.

EXPERIMENT #1: BEHAVIORAL FEVER AND PREFERRED BODY TEMPERATURES

Introduction

If Mormon crickets react to a *B. bassiana* infection with a behavioral fever similar to *M. sanguinipes* (Inglis et al. 1996), then one should detect an increase in the T_{set} of infected crickets. I tested this hypothesis by placing groups of inoculated and control crickets on a thermal gradient. The thermal gradient allowed crickets to choose freely from a linear range of temperatures approximating those found in their natural habitat. I predicted that the mean T_b chosen by inoculated crickets would be higher than in control crickets.

Again, Inglis et al (1996) found the upper thermal limit for *B. bassiana* growth in *Melanoplus sanguinipes* was 35°C. Mormon crickets inoculated and incubated at 37°C had significantly less mycosis than those held at 34°C or less (O'Neill et al. unpublished data). *B. bassiana*'s thermal threshold for growth in Mormon crickets was between 34° and 37°C.

Materials and Methods

In October 1995, I collected Mormon crickets eggs for the thermal gradient experiment by sifting the soil at a known infestation site in Big Horn County, WY. I placed the eggs in moistened vermiculite and refrigerated them at 4°C. In February, I moved the eggs to environmental chambers programmed for 15°C and constant illumination. After they hatched, I moved the nymphs to acetate tubes in environmental chambers programmed for a 25 °C daytime/13 °C nighttime, 14/10 L/D cycle, and fed

them organic Romaine lettuce and wheat bran every 2 days. After the fifth to sixth instar stage, I segregated the nymphs into 1 m³ cages by sex.

At the beginning of each thermal gradient trial, I inoculated half of the crickets with dry conidia of *B. bassiana* GHA in an oil formulation; Mycotech Corporation (Butte, MT) supplied the pathogen (a research grade sample of Mycotrol[®]) and the oil (ES-9501). I applied the oil topically in 0.3 µL droplets behind the pronotum, using a Burkard Microinjector equipped with a 50 µL Hamilton Microsyringe (#705) and a 21 ga needle. The spore concentration was 1.0×10^6 conidia/cricket for nymphs and $2.5 - 5.0 \times 10^6$ conidia/cricket for adults. Crickets in control groups received identically-sized oil droplets lacking conidia. Following inoculation, I separated crickets by pathogen treatment and sex (adults only) and placed them overnight in a darkened environmental chamber set at 25 °C. I separated the adult sexes to prevent reproductive behavior from interfering with thermoregulation.

The thermal gradient consisted of four parallel aluminum bars 107 cm long, 15 cm wide and 0.8 cm thick placed 5 cm apart on a bed of polystyrene foam. The bars' ends rested on four pieces of 2.5 cm square aluminum tubing 87 cm long, two at each end perpendicular to the long axis of the bars and recessed in the foam. Pumps circulated water from water baths through the square tubing and cooled the bars to 15 °C at one end and heated the other to 60 °C. I taped 40 ga. copper-constantan thermocouples to the bars at 10 cm (1 bar) or 20 cm (3 bars) intervals and measured T_s with a digital thermometer (Cole-Parmer, No. 8528-20). Plexiglass enclosures 2.8 cm high covered each bar. A cardboard enclosure blocked light and deadened room noise. I installed a red incandescent light for use only during observations.

For 4 days after their inoculation, I placed 25 4th to 5th instar or 15 adult Mormon crickets on each gradient bar. I mapped the position of each cricket and recorded T_s at 1, 2, and 4 hours after placement. I did not record the position of crickets that moved within

one minute prior to the observation. After completion of observations on the gradient each day, I returned the crickets to the environmental chamber. I conducted three nymphal and three adult trials, each consisting of two replicates of inoculated and two of control crickets randomly assigned to one of the four bars. I cleaned the gradient bars and covers with a bleach solution after each day's observation to prevent inoculation of the controls.

The temperature gradients measured on the four bars differed slightly from each other during the four hours of observation on a single day. The T_s gradients differed more across the four days in a trial and between the six trials. Therefore, a cricket's position on a bar was not a reliable comparison of temperature choices because the T_s (and the cricket's T_b) associated with a bar position (in cm) was not the same across bars, days, or trials. By comparing cricket T_b instead of position, I could account for the variation between bars in T_s . I estimated the T_b of crickets in the gradient by using cricket models: dead crickets with 30 ga. thermocouples implanted midway through the thorax. I placed each of eight freshly killed models, four nymph and four adult, directly over each of the 11 thermocouples measuring T_s on one of the bars. I arranged the models in the same posture that stationary crickets normally exhibited on the gradient: ventral surface of thorax and abdomen touching the gradient bar. After equilibrating for 20 minutes I recorded the model's temperature (T_e) and the T_s at its location. I repeated this procedure after the models had dried for one month to account for effects due to loss of water content and weight. A reliable linear relationship existed between T_e and T_s ($T_e = 6.67 + 0.74T_s$, $r^2 = 0.99$).

I used the T_s data collected during the mapping of a bar to calculate the T_s under each cricket on that bar using the regression of T_s on thermocouple position ($r^2 > 0.94$ for each of 276 bars). Then, using the T_e equation, I estimated a T_b for each Mormon cricket on a bar. I discarded all data for crickets which were on the walls or ceiling of the Plexiglass

cover because I could not accurately determine the T_s in those positions. I also discarded data for molting nymphs after observing that they usually moved to the cool end of the gradient during a molt. To check the assumption that a Mormon cricket's position choice was based on temperature, I placed live crickets on the gradient bars without a temperature gradient in place. When no light sources were present the crickets did not congregate or show a location bias on the bars.

I diagnosed crickets for *B. bassiana* mycosis by sealing them in 25 ml plastic cups with a damp cotton ball immediately after death. Five to eight days later I assessed *B. bassiana* colonization by the appearance of characteristic hyphal blooms on a cricket cadaver (Tanada and Kaya 1993). Five percent of the control crickets that died during the experiment were diagnosed with a *B. bassiana* infection compared to 50% for the inoculated treatment. There was no replacement of crickets during the experiment to compensate for mortality.

The factors in the data analysis were pathogen, age/sex, and days after treatment. I conducted separate analyses for each observation period (Hours 1, 2, and 4) with PROC MIXED (SAS Institute, 1997) using days after treatment as a repeated measure factor. The response used in all analyses was the mean T_b of the crickets on a single bar at a single observation time. I calculated the preferred temperature range (T_{set}) by finding the bounding values of the middle 50%, or the first and third quartiles, of the T_b 's for each bar (Hertz et al. 1993). The means of these values were the upper and lower values of T_{set} for each age/sex*pathogen group.

Results

I recorded 3,845 observations on 301 nymphs and 193 adults from March to May 1996. The resulting data contained mean temperatures for 70 bars (22 nymph, 24 adult female, 24 adult male) at each of the three observation times. The PROC MIXED analysis

showed that many of the interaction effects in the model were significant. I used linear combinations of factor effects to test many hypotheses, including: 1) differences between inoculated and control treatments on specific days post-inoculation, 2) interactions among age/sex levels and day after inoculation, and 3) differences between inoculated and control treatments by age/sex group. However, I could not find any interaction effects in the data which were significant and biologically meaningful. The pattern of temperature choices over days after inoculation were inconsistent between observation times which indicated to me that any effects for days after inoculation were not reliable.

The main effects from the analysis are shown in Table 1. The pathogen main effect was significant in Hours 1 and 2, and the T_b of inoculated crickets was higher than controls at all three observation times. The age/sex main effect was highly significant at all observation times, and the adult males consistently had a lower mean T_b than the nymphs and adult females. The day main effect was significant in Hours 2 and 4, but the pattern of T_b across days was different for each observation time.

Table 1. PROC MIXED results for main effect factors of thermal gradient data by observation time after placement.

Factor	Hour 1	Hour 2	Hour 4
Pathogen	$F_{1,14} = 14.01$ $P = 0.0022$	$F_{1,14} = 5.04$ $P = 0.0414$	$F_{1,14} = 2.13$ $P = 0.1662$
Age/Sex	$F_{2,14} = 68.06$ $P < 0.0001$	$F_{2,14} = 163.88$ $P < 0.0001$	$F_{2,14} = 87.71$ $P < 0.0001$
Day	$F_{3,14} = 2.76$ $P = 0.0814$	$F_{3,14} = 9.51$ $P = 0.0011$	$F_{3,14} = 4.65$ $P = 0.0186$

Inoculated crickets increased their mean T_b by only 0.17°C over controls (Table 2). Male cricket T_b was 1.65°C lower than nymphs and adult females combined. T_{set} range was 0.85°C narrower in nymphs than adults. The range of T_{set} in Hour 4 is 0.52°C

smaller than in Hours 1 and 2 for adults, but the width of the nymph T_{set} is similar across observation times. The mean T_b for each group was relatively constant across the three observation times.

Table 2. Body temperature (T_b) and preferred body temperature range (T_{set}) of Mormon crickets on a thermal gradient ($N = 33$ for nymphs and $N = 36$ for adults) averaged across observation times. Letter beside age/sex class indicates treatment with *B. bassiana*: C is control, I is inoculated.

Age/Sex		T_b ($^{\circ}\text{C}$) (SE)	T_{set} ($^{\circ}\text{C}$)		
			Lower Bound	Upper Bound	Range
Nymph	C	36.28 (0.17)	35.57	37.17	1.60
	I	36.38 (0.15)	35.82	37.12	1.30
Adult Female	C	36.62 (0.24)	35.62	37.99	2.37
	I	36.88 (0.22)	35.91	38.35	2.44
Adult Male	C	34.81 (0.12)	33.89	36.16	2.27
	I	34.97 (0.14)	34.00	36.14	2.14

Discussion

Mormon crickets inoculated with *B. bassiana* showed a slight, but consistent, behavioral fever on a thermal gradient. The fever response was a small increase in the T_b of inoculated crickets of each age/sex group over the controls of that group. In another possible fever response, crickets might have elevated T_b to a similar febrile level for all age/sex groups. For example, instead of increasing T_b by 0.16°C above their normal T_{set} , inoculated males would have increased their T_b by 1.82°C matching the mean febrile T_b chosen by nymphs and adult females. Unfortunately, I cannot report which response benefits the host more, elevation of T_b over normal T_{set} or the absolute febrile T_b reached. Experiments using organisms with variable T_{set} 's (seasonal variation, sexual differences, etc.) could reveal if different nonfebrile T_{set} 's affect the absolute temperature of a behavioral fever.

The magnitude of the fever found here was small compared to the fever responses of other insects: 2.2 - 9.2°C in a cockroach (Brönstein and Conner 1984) 6.4°C in a cricket (Louis et al. 1986), 6.3°C in a grasshopper (Boorstein and Ewald 1987) and 1.5°C in a beetle (McClain et al. 1988). However, Mormon crickets may not need a behavioral fever to resist mycosis by *B. bassiana*. The T_{set} of control crickets was near or above the 35°C upper thermal limit of *B. bassiana* growth.

The significance of such a small increase in T_b as a behavioral fever is questionable. It is doubtful that a Mormon cricket can distinguish body temperature differences as small as 0.17°C in the field. It is also unlikely that fungal growth would be negatively impacted by such a small change in the host's T_b . A logical follow-up experiment after finding evidence of a behavioral fever is to incubate inoculated crickets at normal and febrile temperatures and observe differences in the mycosis and mortality rates. But this experiment is not realistic given that the stated accuracy of most growth chambers is $\pm 0.5^\circ\text{C}$.

The difference between male T_{set} and those of nymphs and adult females exceeded differences due to pathogen treatment. In this experiment, all crickets were fed a low-protein diet and the sexes were segregated during incubation and on the gradient. The sexual difference in T_{set} 's found here may be an artifact of those rearing conditions. As adults, male Mormon crickets produce large, proteinaceous spermatophores and present them to the female when mating. In research using a similar low protein diet, few of the males produced spermatophores (Gwynne 1993). Females stressed by the low protein diet supplemented it by trying to obtain more spermatophores with an increased mating frequency. However, a low protein diet caused sexual selection by males on potential mates based on weight. Females, therefore, might maintain a relatively higher T_b to increase net energy intake and body weight (Harrison and Fewell 1995). For males, a lower T_b could decrease their metabolic rate and conserve resources (body fat or a

completed spermatophore) until conditions improve (a better diet or the appearance of females for mating).

Using the variance of T_{set} as a measure, Mormon cricket nymphs thermoregulated more precisely than adult females or adult males ($P < 0.0001$ for both). However, the difference between nymphs and adults is not entirely behavioral. The smaller body size of the nymphs allowed them to form denser groups around their preferred T_b than the larger adults. Fourth and fifth instar nymph body length is approximately 35% of the length of adult female and male Mormon crickets (Pfadt 1994).

EXPERIMENT #2: MORMON CRICKET BEHAVIORAL THERMOREGULATION

Introduction

Mormon crickets cope with the greater temporal and spatial variation in microclimate in their natural habitat by using a more complex set of behaviors to regulate T_b than needed in the simple environment of a thermal gradient. A cricket's choice of posture, orientation, and microhabitat over the course of a day are vital components in maintaining a stable T_b . I observed Mormon cricket behavior in the field and sampled T_b 's to quantify the relationship between behavior and T_b . This also allowed me to test the assumption that Mormon crickets could regulate T_b in their natural habitat at levels detrimental to *B. bassiana*. Sampling cricket T_b 's in the field across a wide range of environmental temperatures was an excellent way to validate that assumption.

I hypothesized that Mormon crickets would maintain a T_b within their preferred temperature range when environmental conditions permitted. When environmental temperatures were low I expected to find Mormon crickets maximizing heat gain by behavioral means until they had reached their T_{set} . I then expected the crickets to maintain T_b within the narrow range of their T_{set} despite fluctuations in the thermal environment.

Materials and Methods

I returned to the infestation site in Big Horn County, WY for four days in June and July 1996. I observed and recorded each cricket's location and behavior before capture with my gloved hand. I measured cricket T_b by inserting a 0.8 mm diameter hypodermic

thermocouple probe 4 mm through the mesopleuron into approximately the middle of the thorax. The temperature, as measured on a digital thermometer, stabilized quickly, and readings were complete in less than ten seconds. During the two June sampling dates, a datalogger recorded solar radiation and T_s in the sun and shade every 15 minutes. I used a thermocouple and digital thermometer to measure T_s in the sun every 15 minutes during the July observations.

I classified cricket behavior into five categories: perching on a sage (*Artemisia* sp.), walking, basking, normal, and beneath a sage. A basking cricket was oriented perpendicular to the sun's rays and had usually lowered its sunward leg and rolled slightly onto the side away from the sun. The normal posture of a Mormon cricket was with the body held level and close to the ground. Crickets were beneath a sage if they were around the base of a sagebrush plant but were not perching in its branches. For each observation, I also recorded the insolation at the cricket's location: full sun, partial shade, or full shade.

To combine data across days sampled, I calculated a T_s value for each T_b observation, rather than using the time of observation. I analyzed the relationship between T_b and T_s using nonlinear regression to estimate the parameters of a segmented model (O'Neill and Kemp 1990). The segmented model fitted different linear regressions to the data before and after a break point (α). The break point was interpreted to be the T_s at which Mormon crickets switched from behaviors that increased T_b to ones that kept T_b near T_{set} . In this experiment, the regression equation below α was $T_b = a + b(T_s)$, and above α the slope of the regression line was defined as zero ($T_b = a$).

I analyzed behavioral and insolation data as a proportion of all observations in one of six T_s categories from 10 - 50°C. Assuming that sampling was independent of T_s , I used the χ^2 method of Fleiss (1981) to make comparisons among groups suggested by the data when an overall χ^2 test was first significant. I used Tukey's tests in each T_s category to compare T_b 's for each behavior and insolation level.

Results

Mormon cricket T_b ranged from 8.8 - 42.0°C in the 1,034 samples taken over a T_s range of 10.5 - 49.7°C (Figs. 1-4). On June 27, midday clouds interrupted a steady increase in T_s (Fig. 1). Earlier that morning several Mormon crickets had T_b 's several degrees higher than T_s . Before sunrise on June 28, Mormon cricket T_b did not differ much from T_s (Fig. 2). I stopped taking data when heavy clouds moved in just at dawn. There was no direct sunlight during the time of data collection. Measurements taken on a hot and cloudless July 22 afternoon showed that Mormon crickets maintained their T_b near T_{set} despite high T_s (Fig. 3). The morning of July 23 was also cloudless and some Mormon crickets raised their T_b above T_s in the early hours (Fig. 4).

The segmented regression showed that there was a linear relationship between Mormon cricket T_b and T_s when $T_s < \alpha$ (Table 3, Fig. 5). When $T_s > \alpha$, the slope of the regression line was not different than zero (female: $F_{1, 254} = 2.67$, $P = 0.10$; male: $F_{1, 185} = 0.17$, $P = 0.68$). The female T_b when $T_s > \alpha$ (35.9°C) fell within the female T_{set} (35.6 - 38.0°C), but the male T_b (36.3°C) was not included in its T_{set} (33.9 - 36.2°C). The percentage of T_b observations that fell within their T_{set} 's when $T_s > \alpha$ was 41.2% for females and 30.6% for males.

Table 3. Segmented regression of Mormon cricket T_b samples.

	T_s at α $\pm$ SE (°C)	Pre - α	Post - α
Female	36.64 $\pm$ 0.31	$T_b = 1.40 + 0.94T_s$ $F_{1,272} = 2817.6$, $P < 0.0001$	$T_b = 35.93^\circ\text{C}$ SE = 0.14, N = 255
Male	37.37 $\pm$ 0.28	$T_b = 1.19 + 0.94T_s$ $F_{1,319} = 3311.7$, $P < 0.0001$	$T_b = 36.31^\circ\text{C}$ SE = 0.16, N = 186
Both	37.03 $\pm$ 0.21	$T_b = 1.35 + 0.94T_s$ $F_{1,592} = 6236.7$, $P < 0.0001$	$T_b = 36.10^\circ\text{C}$ SE = 0.11, N = 441

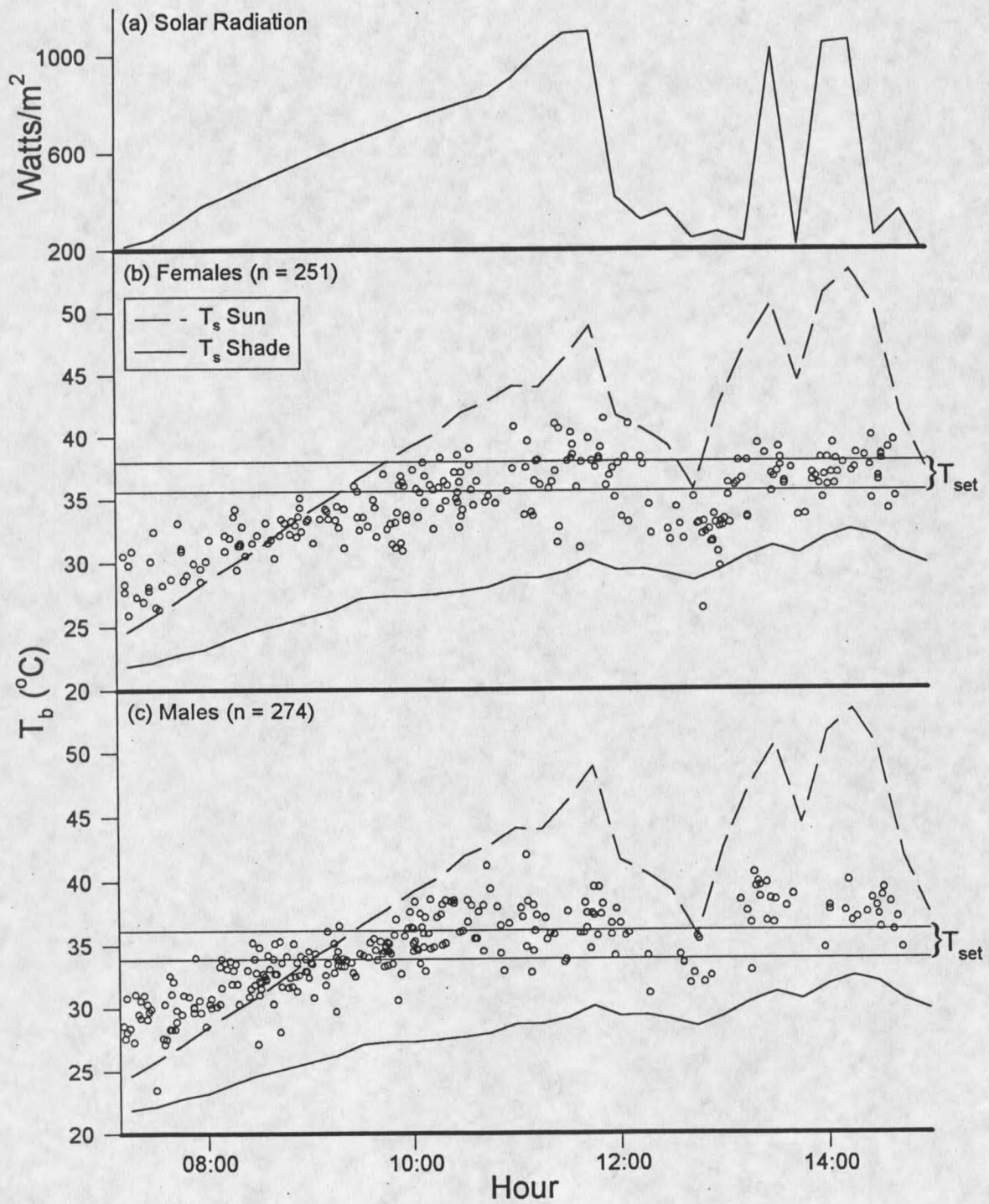


Figure 1. Solar radiation levels (a) and Mormon cricket body temperatures (b, c) on June 27, 1996.

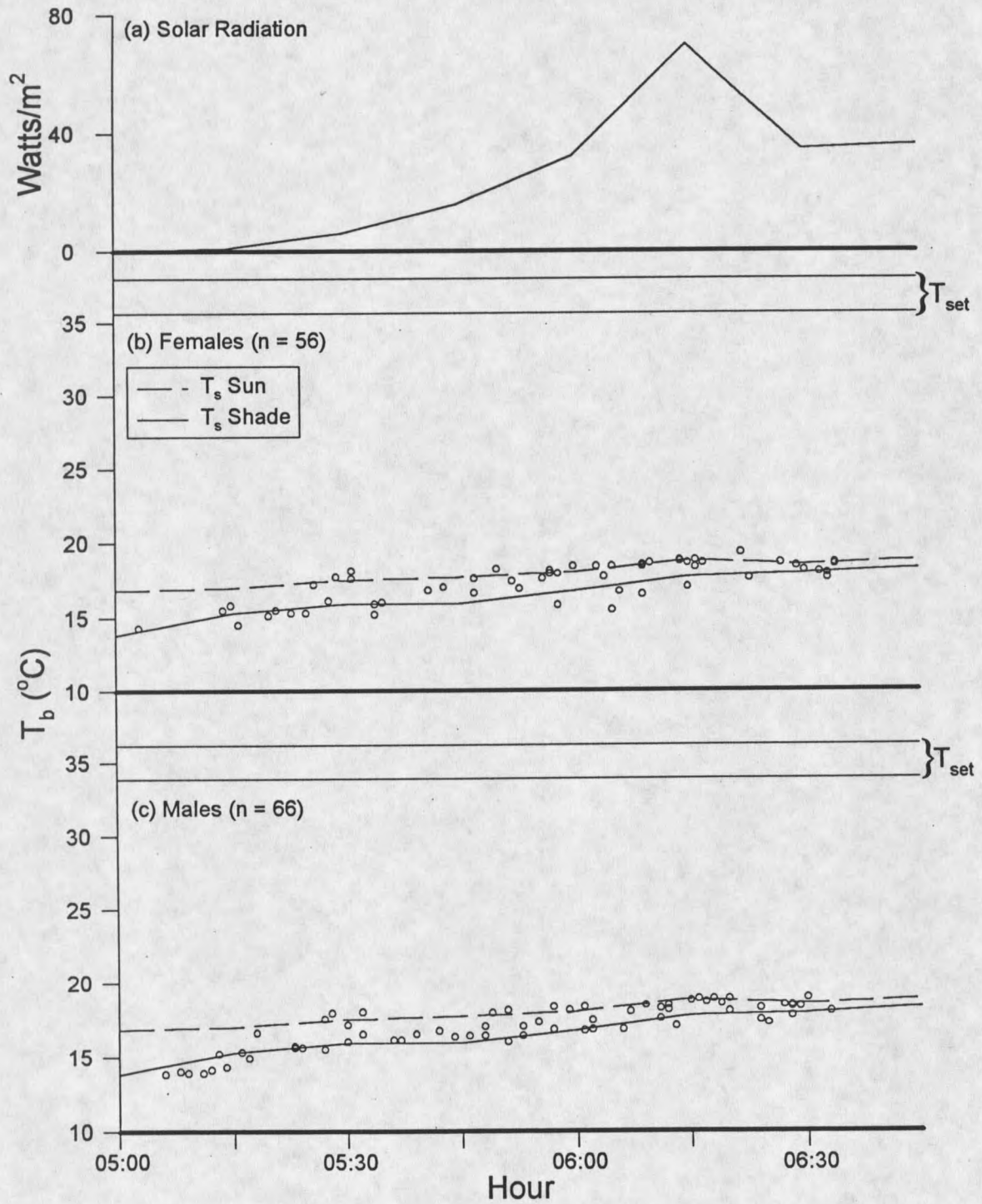


Figure 2. Solar radiation levels (a) and Mormon cricket body temperatures (b, c) on June 28, 1996.

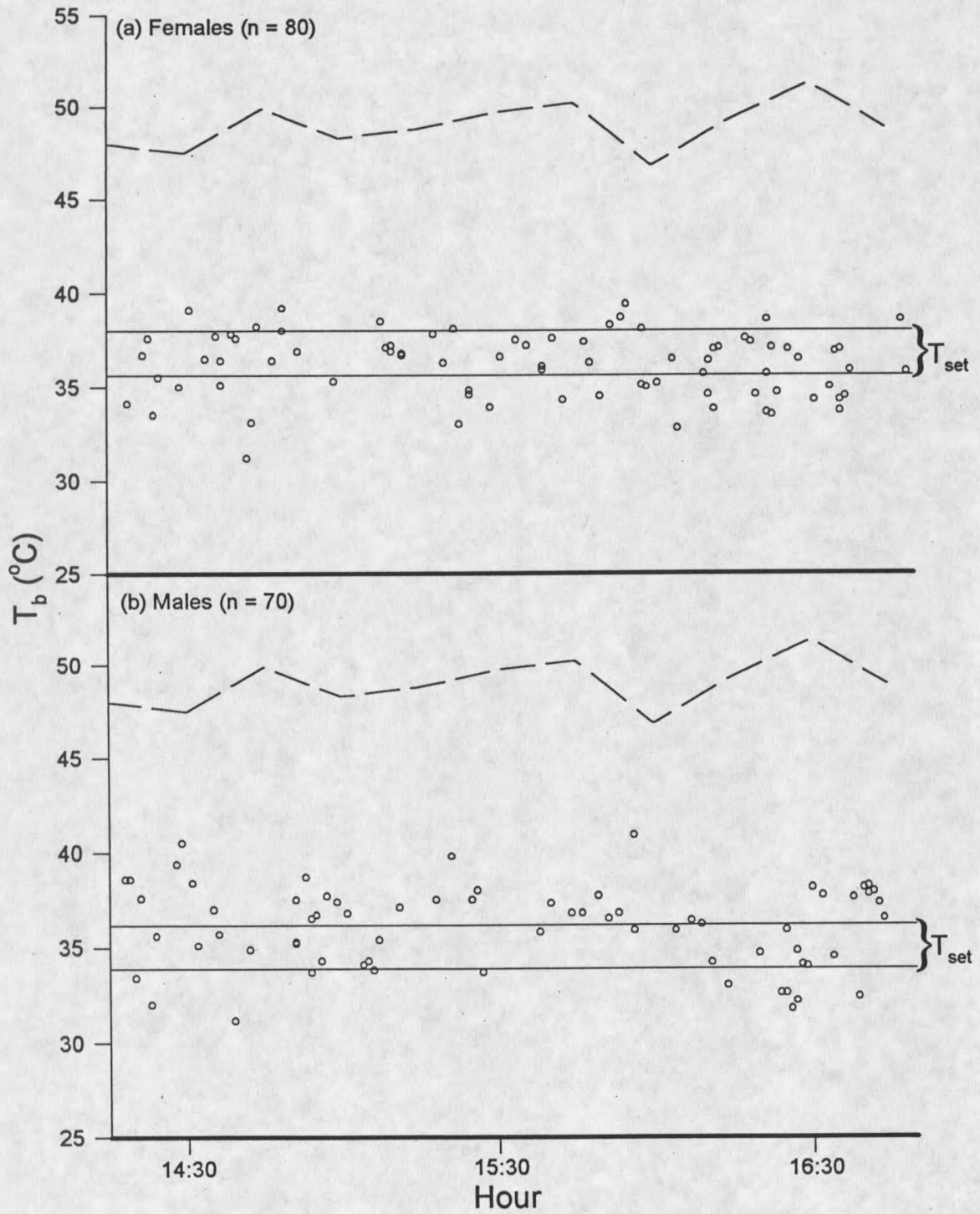


Figure 3. Mormon cricket body temperatures on July 22, 1996. Dashed line indicates T_s in the sun.

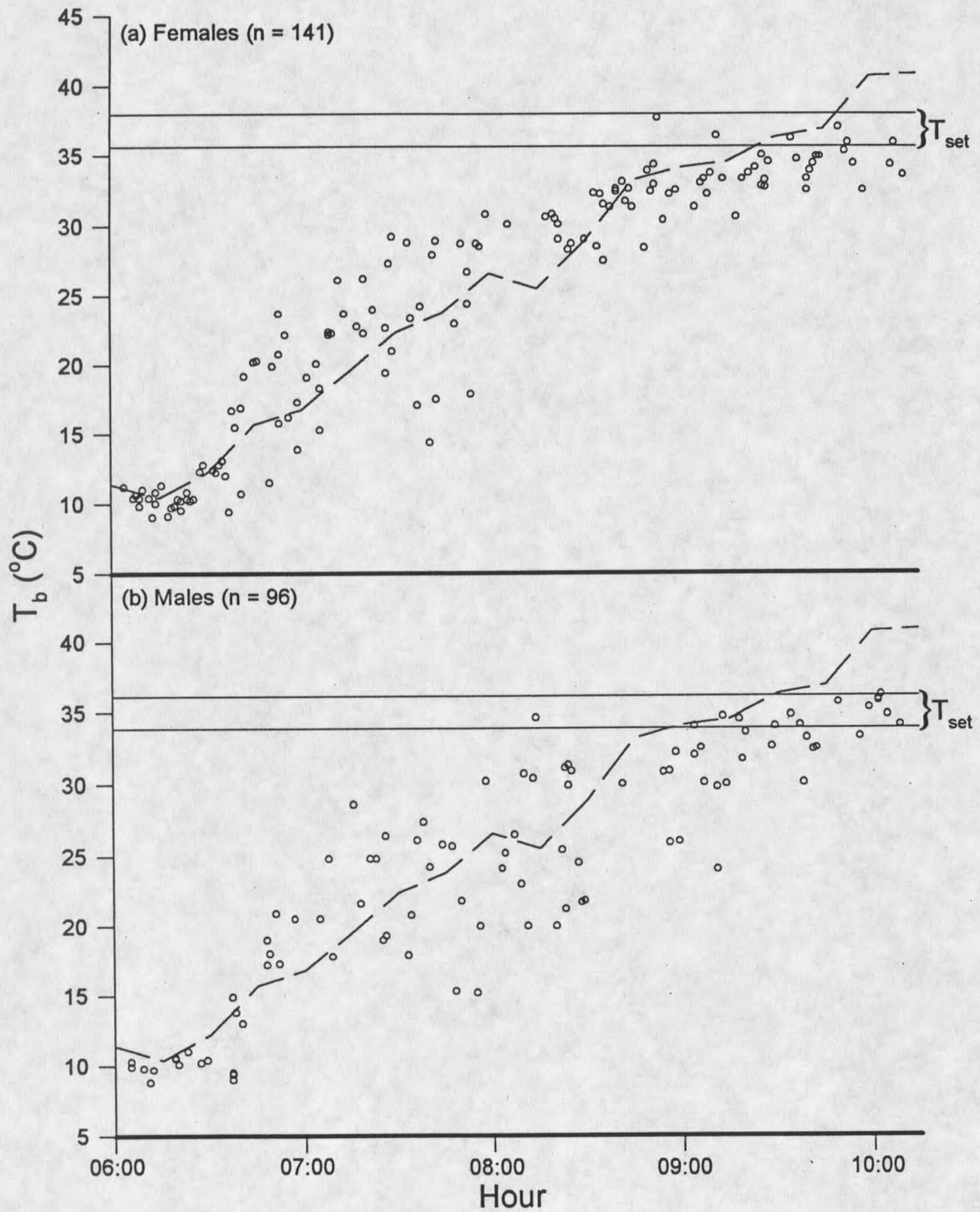


Figure 4. Mormon cricket body temperatures on July 23, 1996. Dashed line indicates T_s in the sun.

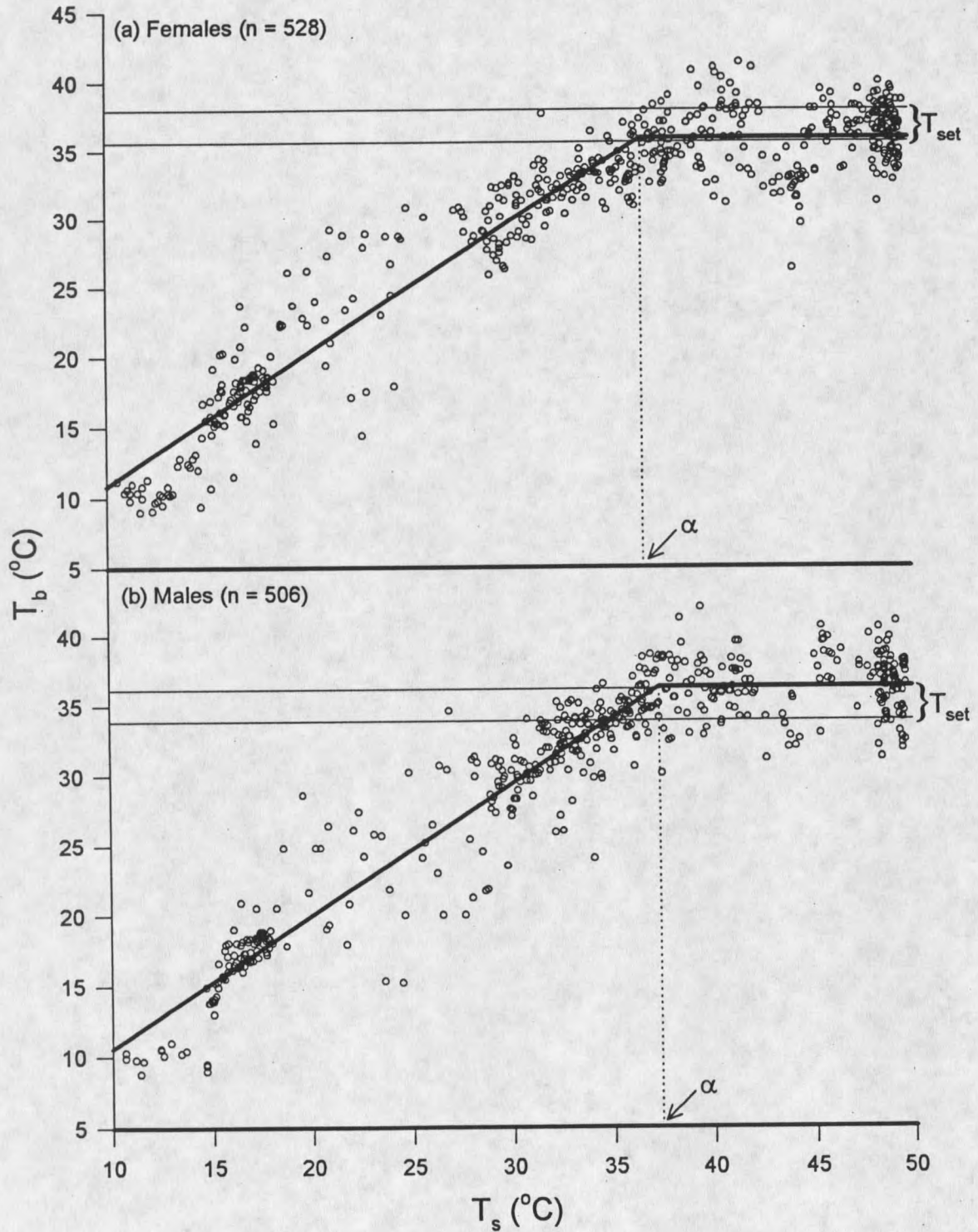


Figure 5. Segmented regression of Mormon cricket body temperatures. α is the T_s at which the two regression segments meet. T_s at $\alpha = 36.6^{\circ}\text{C}$ in (a) and 37.4°C in (b).

The results of a multiple regression on all observations in a segmented model revealed that gender was not a significant factor in explaining the variation in T_b (before α : $T = -1.44$, $P = 0.15$; after α : $T = 1.65$, $P = 0.10$). In a single segmented regression model containing male and female observations, when $T_s > \alpha$ the mean T_b was 36.1°C .

In behavioral observations across all T_s categories, crickets were perching (24.2%), walking (29.6%), or stationary and normally postured (28.7%). The remainder were beneath a sage (9.6%), basking (6.2%), eating (1.5%), or stiling (0.3%). Eating and stiling were not included in subsequent analyses because their expected values in a χ^2 analysis were too small to be dependable. I did not sample cricket T_b 's in the late afternoon or evening when T_s was decreasing. Therefore, the increase in temperature through the six T_s categories approximates the time period from morning to early afternoon.

Behaviors were nonrandomly distributed across T_s categories (Fig. 6). The proportion of Mormon crickets perching on plants and walking was least when T_s was $30 - 39^\circ\text{C}$. Mormon crickets assumed the normal posture on the soil surface more often when T_s was $35 - 44^\circ\text{C}$, and were found beneath a sage most often when T_s was the coolest and hottest. Basking occurred most often from $20 - 34^\circ\text{C}$ T_s , and was not observed at $T_s > 40^\circ\text{C}$.

I omitted 175 observations from the insolation data to exclude periods when the sun was obscured and a choice of different locations varying in insolation was not possible (i.e., before sunrise or heavy cloud cover). Of the remaining 859 observations, 26.2% of the Mormon crickets were in full shade, 14.0% were in partial shade, and 59.8% were in full sunlight. The proportion of Mormon crickets in the full shade was greatest when $T_s > 45^\circ\text{C}$ (Fig. 7). There were more Mormon crickets in partial shade when $T_s > 40^\circ\text{C}$, but fewer in the full sun when $T_s > 40^\circ\text{C}$.

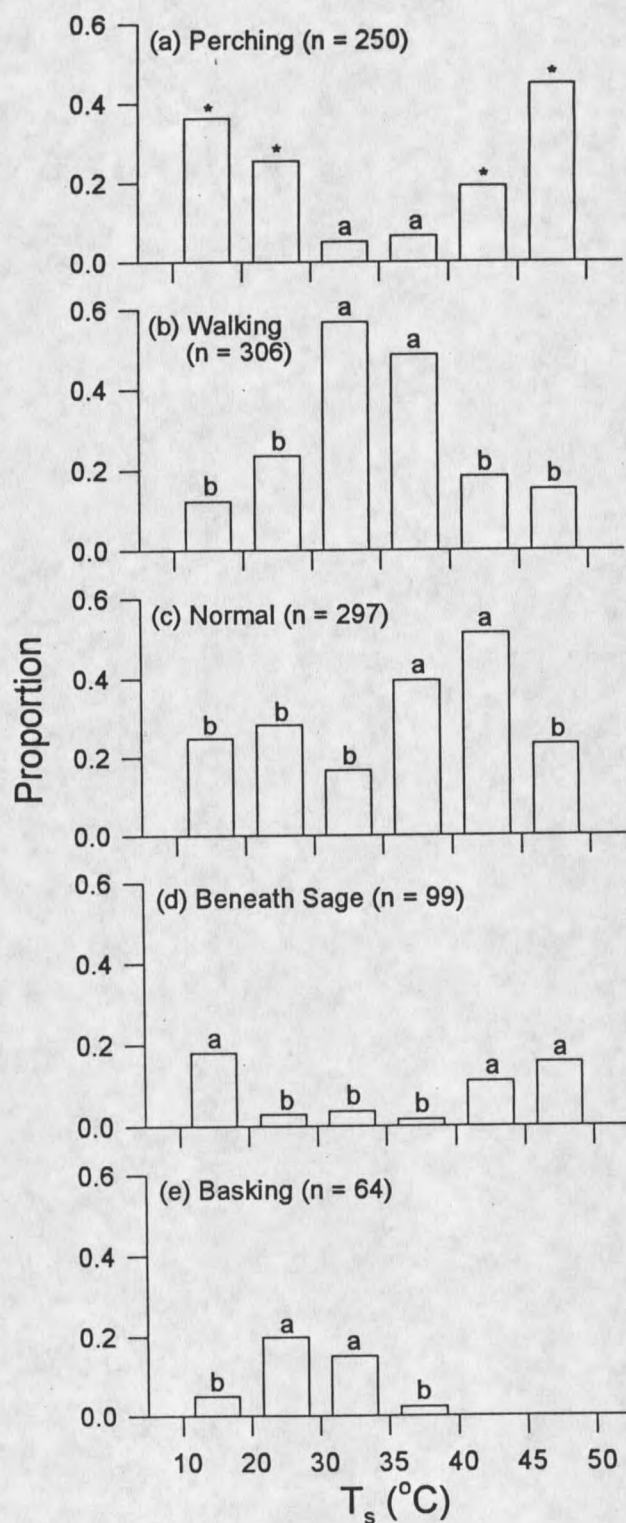


Figure 6. Proportion of Mormon cricket behaviors across T_s categories. Means with different letters are different at $P = 0.05$ (grouped χ^2 test). * denotes values whose group $P > 0.05$. Proportions sum to one in each T_s category.

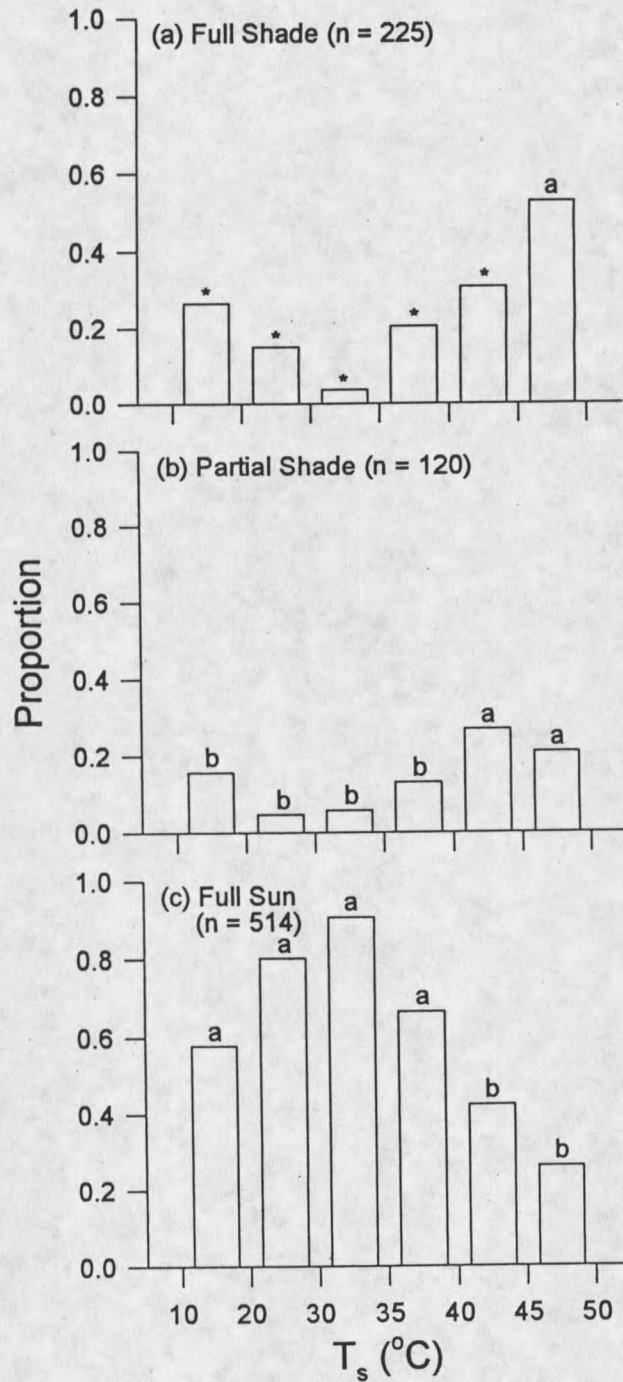


Figure 7. Proportion of Mormon cricket insolation choices across T_s categories. Means with different letters are different at $P = 0.05$ (grouped χ^2 test). * denotes values whose group $P > 0.05$. Proportions sum to one in each T_s category.

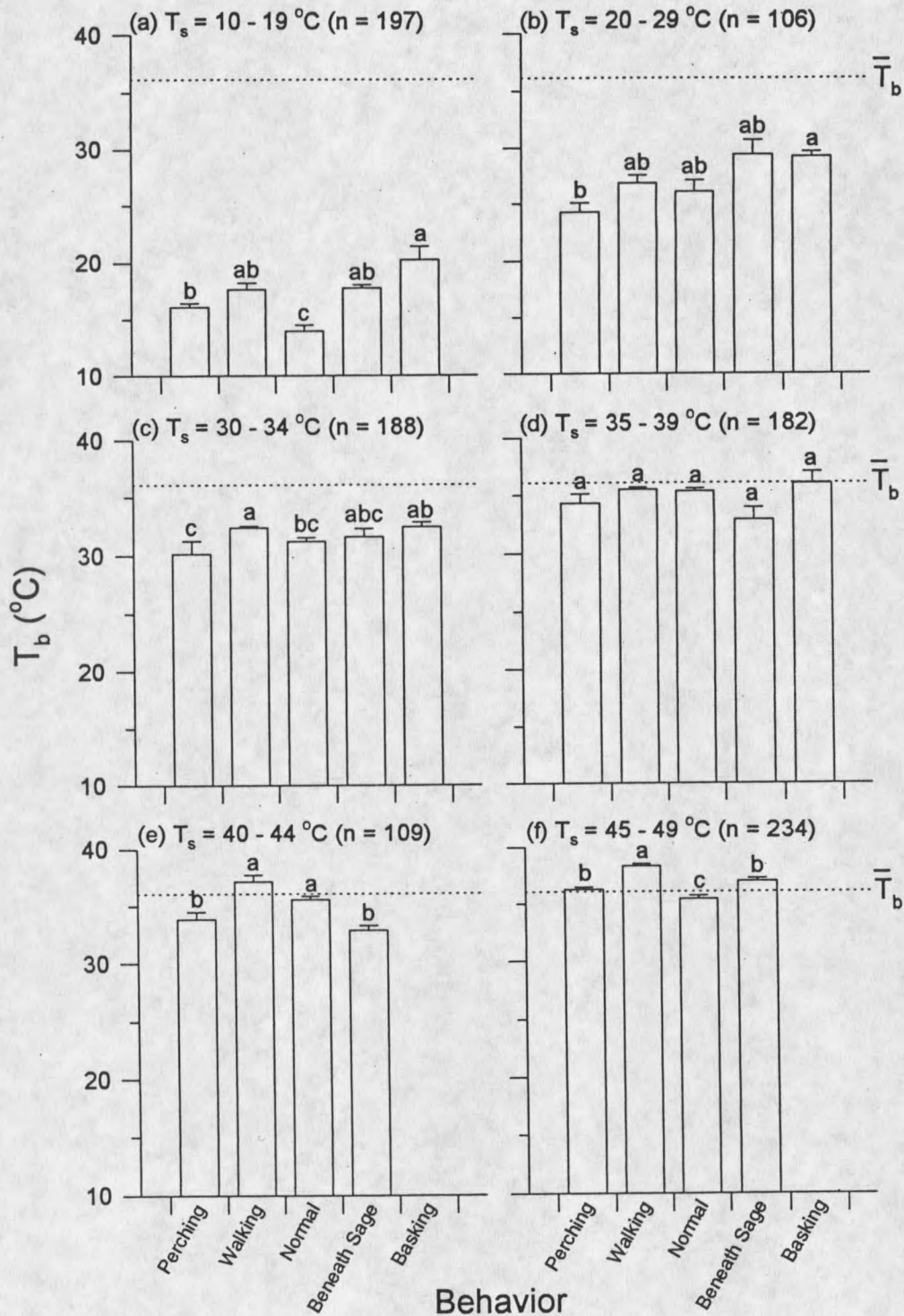


Figure 8. Mormon cricket T_b for behaviors across T_s categories. Means with different letters are different at $P < 0.05$ (Tukey's test). The horizontal dotted line is the mean T_b for all observations when $T_s > 36.1^\circ\text{C}$.

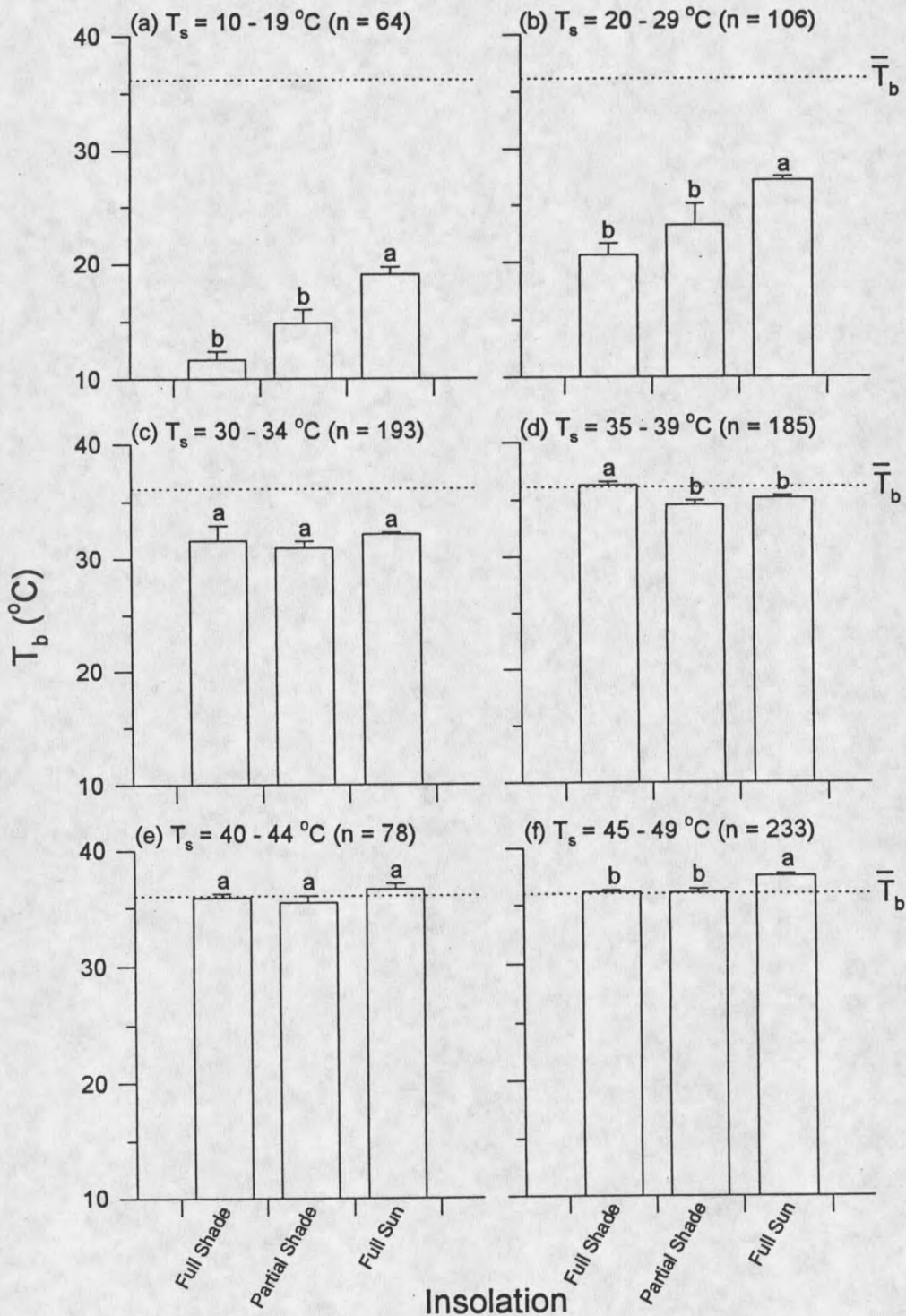


Figure 9. Mormon cricket T_b for insolation choices across T_s categories. Means with different letters are different at $P < 0.05$ (Tukey's test). The horizontal dotted line is the mean T_b for all observations when $T_s > 36.1^\circ\text{C}$.

Within T_s categories, T_b varied within behavior (Figs. 8, 9). Basking crickets had the highest mean T_b when T_s was 10 - 29°C. Walking crickets had some of the highest mean T_b 's when $T_s > 30^\circ\text{C}$. Crickets that were perching or beneath a sage had lower temperatures when $T_s > 40^\circ\text{C}$. Mormon crickets in the full sun had significantly higher mean T_b 's when T_s was 10 - 29°C, and mean T_b increased stepwise from the lowest insolation level to the highest at those temperatures. In the T_s categories $> 30^\circ\text{C}$, mean T_b did not differ by more than 1.7°C between the three insolation levels.

Discussion

The Mormon crickets' thermoregulatory behaviors allowed them to quickly reach and maintain a stable T_b near T_{set} in their natural setting. In the morning, crickets increased their T_b at nearly the same rate as the rise in T_s . When T_b and T_s neared T_{set} , Mormon crickets stopped increasing T_b , so that their T_b was then independent of further changes in T_s and was centered around T_{set} . Body postures and insolation choices shifted from heat-gaining to heat-avoiding ones as T_b and T_s levels rose to T_{set} .

The patterns of Mormon cricket T_b across time (Figs. 1 and 4) and across changes in T_s (Fig. 5), were typical for a thermoregulating organism. The slopes for the segmented regressions before the breakpoint, α , are nearly 1 suggesting that Mormon cricket T_b 's conformed to the highest T_s available. The slopes of the lines after α were no different than zero. This indicated that crickets no longer conformed to the hottest part of their thermal environment (Hertz et al 1993). However, Hertz et al. (1993) discounted the use of regression slopes as a reliable method to judge thermoregulatory behavior. Their preferred method compares T_e 's from randomly placed models and simultaneously sampled T_b 's to the laboratory T_{set} . This method computes an E index to describe the difference between a thermoregulating organism and one responding randomly to its thermal environment.

The lack of T_e model data and an E index does not decrease the usefulness of the data presented here. Christian and Weavers (1996) found the E index did not provide any more information than could be found by examining plots similar to Figures 1 - 4. Thus the comparison of T_b 's available to an organism to the T_b 's actually chosen by the organism can be done visually. In Figure 1, it was clear, that at times when the T_s range overlapped T_{set} , many of the crickets had T_b 's within T_{set} . Before T_s rose above the lower T_{set} value, or when cloud cover lowered T_s below T_{set} , few crickets had a T_b within T_{set} . Figure 3 shows that Mormon crickets maintained a T_b centered around T_{set} despite a wider range of temperatures possible in their environment. There can be no doubt that Mormon crickets thermoregulate around a preferred T_b within the constraints of their thermal environment.

It is clear that Mormon crickets thermoregulate behaviorally. They were found more often in the sun basking when T_s was low, and they sought shade and partial shade at high T_s . Crickets perched or were found beneath a sage at high and low T_s , and they walked most when T_s was near T_{set} .

The proportion of crickets walking was highest during midmorning when T_s was 30 - 39°C (Fig. 8b). At those temperatures, the T_b of walking crickets was closest to T_{set} or did not differ from the T_b of other behaviors (Figs. 8c and 8d). The population of Mormon crickets studied here were not migrating, but they followed the daily movement pattern reported for a migrating band by Cowan (1990). Crickets began migrating when T_s reached 30°C. Above 41°C, the crickets stopped moving and roosted in bushes until temperatures decreased.

Mormon crickets seek shelter in plant canopies at night (Cowan 1929), and feed in the tops of sagebrush in the evening (Pfadt 1994). Ostensibly, the crickets here remained in the shelter of the plants overnight and were found there in the early morning (Figs. 6a and

6d). Mormon crickets returned to plant cover when $T_s > 40^\circ\text{C}$, presumably to avoid high temperatures.

Although female and male T_{set} 's differed on the thermal gradient, there were no differences between the sexes in T_b in the field. With data from a natural population, it now seems more likely that the sexual differences in T_{set} on the thermal gradient were perhaps a product of the incubation conditions rather than different T_{set} 's for the sexes. The T_b 's found in the field for males and females were closest to the female and nymph mean T_b 's found on the gradient. It appears that the males on the gradient lowered their T_{set} (some possible reasons were mentioned above).

EXPERIMENT #3: FIELD ENCLOSURE MORTALITY

Introduction

Environmental conditions in the field at the time of a *B. bassiana* application could be more important than the behavioral reaction of the Mormon cricket. If low temperatures or cloud cover prevented Mormon crickets from reaching their normal T_{set} , regardless of any increase due to behavioral fever, then mycosis might proceed unchecked by host temperature constraints. I tested whether manipulation of the Mormon cricket's thermal environment would change their susceptibility to the fungus. This was done by preventing one group of Mormon crickets from raising their T_b to levels detrimental to *B. bassiana*, while allowing others to thermoregulate normally at a wide range of temperatures. In the field, I used unshaded and shaded cages as the high (= variable) and low temperature treatments, respectively. The four temperature and pathogen treatments were: 1) control-unshaded, 2) control-shaded, 3) inoculated-unshaded, and 4) inoculated-shaded. I hypothesized that *B. bassiana* mycosis would be greatest in the inoculated-shaded enclosure and that the inoculated-unshaded treatment's mycosis levels would be no higher than that of the controls.

Materials and Methods

I built eight sheet metal cages 1.2 m^2 at the base with inward slanting sides 28 cm high and placed them on weed-proof cloth 1.5 - 2 m apart. After orienting the sides of each cage to the cardinal directions, I leveled their interior floors with 4 - 6 cm of clean sand and then banked sand 20 cm high around the outside of the walls. A thin layer of

petroleum jelly applied to the top of the inside walls of the cages prevented crickets from escaping. After each day's final mortality count, I placed grasses and forbs in each cage for food and covered the cages with aluminum window screen to prevent escape by crickets and entry of predators. I removed any remaining food and the screens each morning. I positioned nine 1.0 x 15 cm dowels marked in 5 cm increments vertically in each cage to provide perches.

I assigned the four northernmost of the eight cages to the shaded treatment and elevated two layers of shade cloth (approx. 8% light transmission) (Easy Gardener, Waco, TX) approximately 30 cm above the tops of the cages. By tilting the shade cloths as the position of the sun changed during the day I could ensure full shade and adequate ventilation in the cages. Also, I did not remove the aluminum window screen covering the tops of the shaded cages during the day.

The unshaded treatment consisted of the remaining four cages. During the day I replaced the aluminum window screen covers with 1.7 x 2.1 cm mesh bird netting which allowed full illumination but prevented predation. If the weather conditions were hot and dry, I watered the banked sand around the outside of the cages in both temperature treatments in the morning. I did not attempt to control humidity in the cages because *B. bassiana* mycosis is independent of ambient relative humidity (Marcandier and Khachatourians 1987). A datalogger (Polycorder 516C, Omnidata International, Logan, UT) recorded T_s in four positions in one cage of each temperature treatment with 24 ga copper-constantan thermocouples every 15 minutes during each day of the first two trials.

I collected 7th instar and adult Mormon crickets from high density populations 15 km east of Greybull, WY and placed them in environmental chambers programmed for a 25°C daytime/20°C nighttime, 12/12 L/D cycle until they were placed in the field cages. I inoculated the crickets using the same procedures described above but changed the droplet size to 0.16 μ L and the spore concentration to 2×10^6 conidia/cricket. One day after

inoculation I randomly assigned crickets to each cage; one cage in each temperature treatment received 1) inoculated males, 2) inoculated females, 3) control males, and 4) control females. Two to three times daily thereafter I collected dead crickets from each cage and diagnosed them for *B. bassiana* mycosis (described above). Only 0.5% of the control crickets were colonized by *B. bassiana*, suggesting either that a few of the control crickets were contaminated during the experiment or that some crickets at the field collection site were infected with the fungus.

I conducted four replicate trials from June 18 to August 13, 1996 at a site near the Montana State University campus in Bozeman, MT. Between trials, I removed screens and shade cloth from the cages allowing predators and ultraviolet radiation to eliminate the remaining crickets and *Beauveria* conidia, respectively (Inglis et al. 1997a).

All data on proportions dead and dead due to *B. bassiana* mycosis were transformed before analysis with a modification of the Freeman-Tukey arcsin transformation (Zar 1996) to remediate nonconstant variance and nonnormality. The age class of crickets used and environmental conditions changed between trials so that I did not consider the trials as replicates of the same experiment. Therefore, I analyzed each of the four trials separately for the final proportion dead and the proportion whose mortality was attributed to *B. bassiana* using two-way ANOVAs with two pathogen treatments and two temperature treatments. I compared significant interaction effects with a Tukey's test.

Results

Final mortality ranged from 8% to 100% in individual cages, and the proportion of mycosis among the dead ranged from 0% in most of the control cages to a high of 72% in one of the inoculated-shaded cages; both proportions varied widely between trials (Table 4). Across all four trials, 73% of the *B. bassiana* mycosis in the inoculated-shaded treatments occurred during the second half of the trial ($\chi^2_1 = 18.6$, $P < 0.000$, $N = 86$).

However, non-fungal mortality in that treatment was not skewed toward the first or second half ($\chi^2_1 = 1.1$, $P = 0.29$, $N = 58$) nor was total mortality in the other three treatments ($\chi^2_1 = 0.69$, $P < 0.41$, $N = 284$).

Table 4. Summary of field enclosure trials. Means with different letters were significantly different at $P < 0.05$ (Tukey's test).

Trial	Length (days)	Crickets per cage	Age	Daily Maximum T_a ($^{\circ}\text{C}$)	Inoculated-Shaded Treatment	
					% Mortality	% Mycosis
#1	8	25	70% 7th 30% Adult	21.9 ± 1.3 a	77.4	67.3
#2	9	25	Adult	29.4 ± 1.0 b	32.0	8.0
#3	14	25	Adult	29.0 ± 0.6 b	76.7	55.0
#4	12	30	Adult	28.6 ± 1.6 b	100.0	27.0

In Trial #1, mycosis was significantly higher in the inoculated-shaded treatment compared to all other treatments (Fig. 10a). Total mortality in the inoculated-shaded treatment was significantly higher than in the inoculated-unshaded treatment, but was not different than either of the control treatments (Fig. 11a). All treatments in Trial #2 had low *B. bassiana* infection rates and total mortality rates, and there were no significant differences between the treatments (Figs. 10b and 11b). As in Trial #1, mycosis in the inoculated-shaded treatment of Trial #3 was significantly higher than the other three treatments (Fig. 10c). Both inoculated treatments had significantly higher total mortality than the controls (Fig. 11c). In Trial #4, the inoculated-shaded treatment had significantly higher rates for mycosis due to *B. bassiana* than the other treatments (Fig. 10d). An unknown fungus whose conidiophores were visually distinguishable from *B. bassiana* appeared in this trial. Mycosis by the unknown fungus was greater in the shaded treatments than the unshaded regardless of *B. bassiana* infection ($F_{1,7} = 17.24$, $P =$

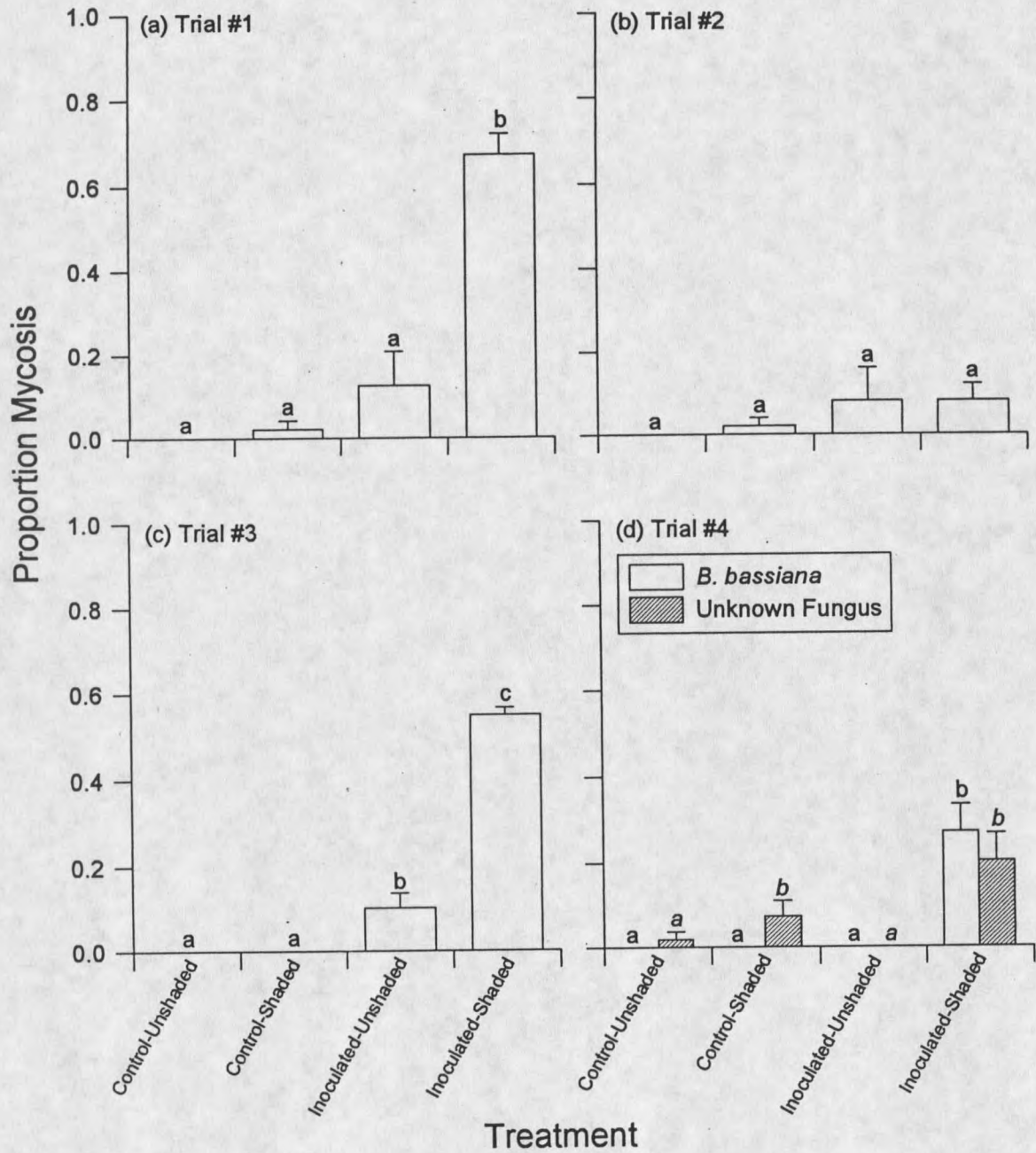


Fig. 10. Final *B. bassiana* mycosis in Mormon crickets under different insolation and inoculation treatments. Means with different letters were different at $P < 0.05$ (Tukey's test) ($n=2$).

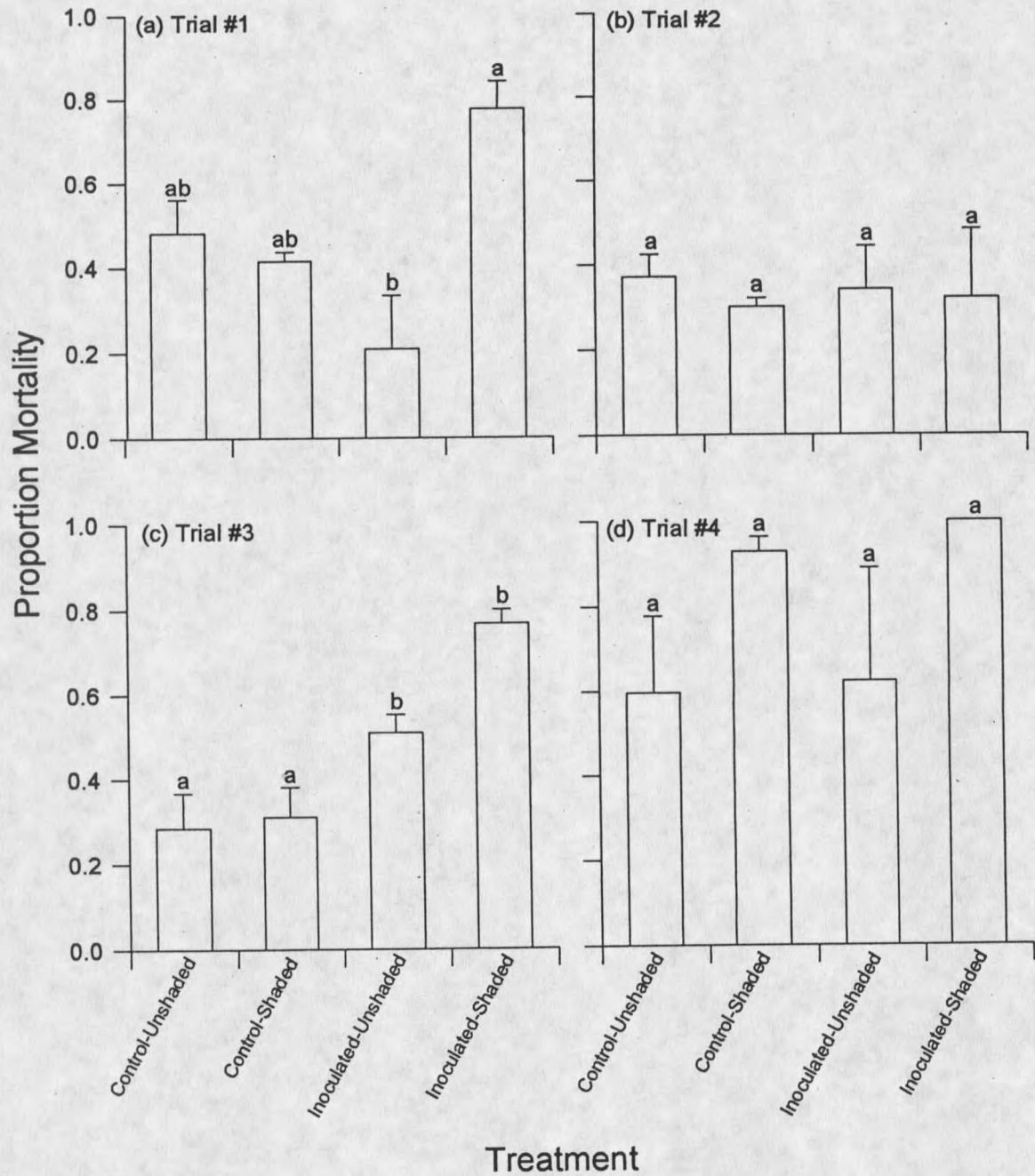


Fig. 11. Final mortality in Mormon crickets under different insolation and inoculation treatments. Means with different letters were different at $P < 0.05$ (Tukey's test) ($n = 2$).

0.014), assuming that all treatments were equally infected. Total mortality rates were high in all treatments and there were no significant differences between treatments (Fig. 11d).

A weather station on the MSU campus 1.5 km away recorded the daily maximum T_a during all four trials. A datalogger recorded T_s in the cages during Trials #1 and #2. The maximum T_a 's reached during Trial #1 were significantly lower ($F_{3,42} = 7.86$, $P < 0.000$) than those in the other three trials (Table 4). The T_s daily maximums recorded by thermocouples in Trials #1 and #2 in a shaded cage were no different ($P = 0.15$, $N = 13$) than the T_a maximums recorded at the weather station. The T_s daily maximums recorded in an unshaded cage during Trials #1 and #2 were 21.6 to 30.0°C (95% CI) higher than the T_a maximums.

Discussion

Trials #1, #3, and #4 are similar to each other and to my expected results. However, the outcome of Trial #2 differed from the hypothesized outcome. I first discuss possible reasons why Trial #2 differed from the other trials.

In Trial #2, mycosis in the inoculated-shaded treatment was only 8% and was no different than any of the other treatments in that trial. Possible explanations are poor germination of the *B. bassiana* conidia, an error in inoculation, delayed onset of mycosis due to temperature, or delayed onset of mycosis due to age. First, the conidial suspension used in Trial #2 was drawn from the same batch used in Trial #1. The inoculum proved effective in Trial #1 and we should expect no difference in the second trial. Though the conidia/oil mix had been refrigerated for 40 days between mixing and its use in Trial #2, *B. bassiana* suspended in oil should maintain at least 90% of its original viability for two years if refrigerated at 4°C (Stefan Jaronski, Mycotech Corp., personal communication).

Second, I inoculated the crickets with the same procedures and equipment as in the other three trials. In one of the twelve replicates of another experiment not reported here,

I found that a syringe needle clogged and did not dispense the inoculum properly, resulting in no mycosis in that replicate. A similar event could have occurred in Trial #2 in this experiment.

Third, higher T_a 's in Trial #2 compared to Trial #1 could delay onset of mycosis beyond the 9 day length of the second trial. The upper thermal limit for germination and hyphal development in *B. bassiana* is approximately 35°C. Although T_s in the shaded treatment never exceeded 35°C it was higher in Trial #2 than in Trial #1. O'Neill et al. (unpublished data) found that temperatures of 30 - 34°C delayed the onset and progress of *B. bassiana* mycosis in Mormon cricket adults. Therefore, higher temperatures could have caused a shift in disease progress.

Fourth, 70% of the crickets in Trial #1 were 7th instars, but the other three trials contained all adults. In a controlled temperature study, O'Neill et al. (unpublished data) found evidence suggesting that the time to 100% mycosis was twice as long in adults as in younger instars. The adult Mormon crickets in Trial #3 took 14 days to reach the levels of infection and mortality found in the mostly 7th instars of the 8 day long Trial #1 (Fig. 12). If mycosis is delayed in adults then the 9 day length of Trial #2 would not capture much of the *B. bassiana* related mortality. However, since I stopped Trial #2 after 9 days I cannot determine if the results would have been different.

In retrospect, I should have performed two checks during every field trial. I would have tested the germination of the inoculum on agar plates and its pathogenicity on Mormon crickets, both held in laboratory incubation. If I had taken these steps I might have eliminated some uncertainties about anomalies in all trials.

In Trial #4, the combination of two factors likely caused high mortality, but not high *B. bassiana* mycosis, across all treatments. I began Trial #4 on August 2 near the end of the adult's life cycle (Wakeland 1959). I also found a fungus other than *B. bassiana* causing mortality in five of the eight replicates in three treatments. The stress associated

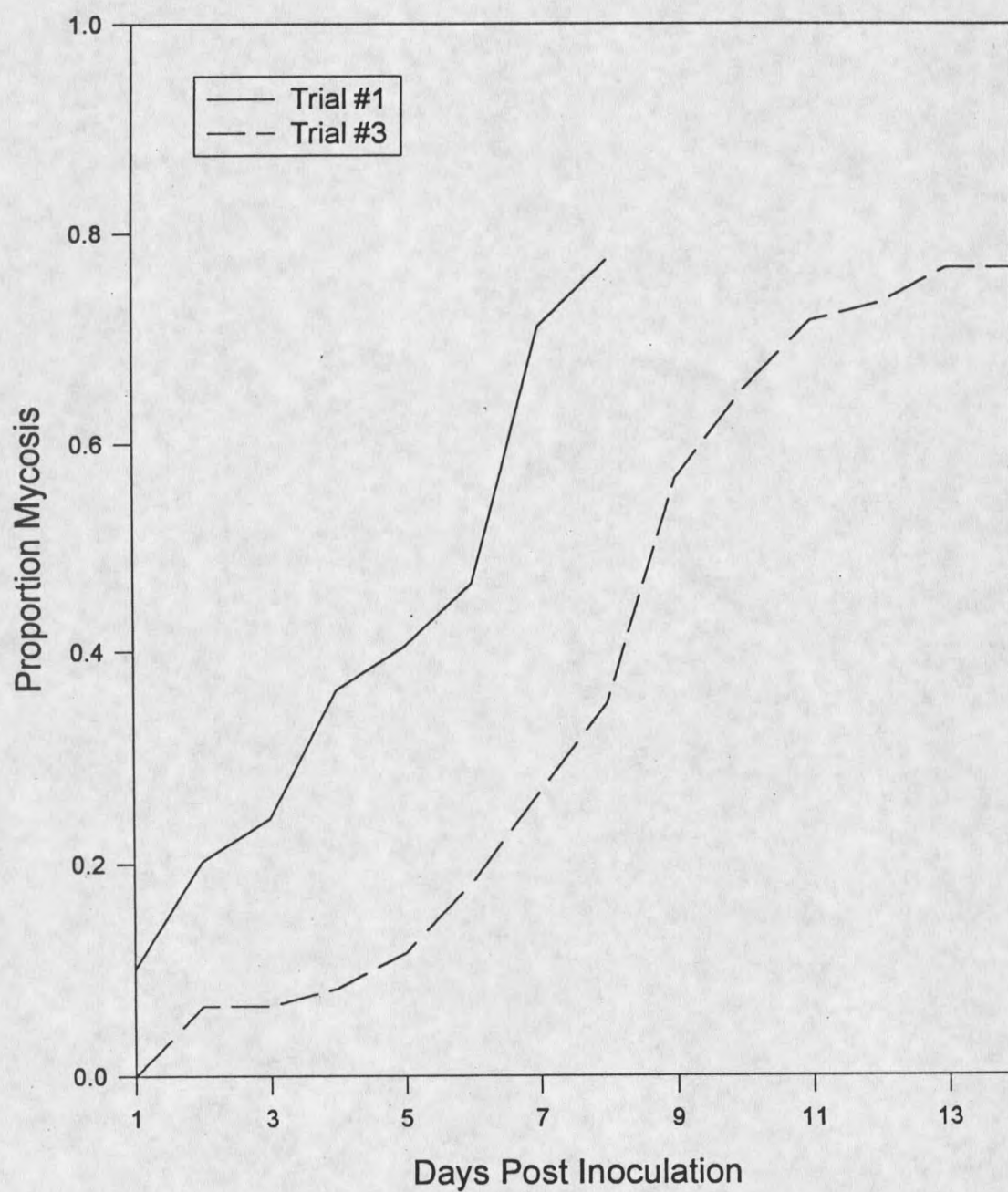


Fig. 12. Progress of *B. bassiana* mycosis in Mormon crickets in the inoculated-shaded cages of Trials #1 and #3.

with these conditions might cause the high mortality found in the trial and the low proportion of crickets diagnosed with *B. bassiana* in the inoculated-shaded treatment. Mycosis from the unknown fungus occurred only in crickets that died during the first 6 days of the trial. Crickets diagnosing positively for *B. bassiana* mycosis occurred only from day 7 onward.

Trials #1 and #3 provide good evidence for my hypothesis that Mormon crickets can resist *B. bassiana* mycosis when allowed access to a normal range of field temperatures. In those trials, the *Beauveria* mortality was significantly higher in the inoculated-shaded treatment than in the inoculated-unshaded treatment. Also, total mortality was highest in the inoculated-shaded treatments. In Trial #4, the *B. bassiana* infection pattern was consistent with this hypothesis although the absolute proportions were lower than in Trials #1 and #3. The infection pattern of the unknown fungus also supports this hypothesis because the lower temperatures in the shaded treatment were conducive to its mycosis. Inglis et al. (1997a) also found that exposure to full sunlight reduced *B. bassiana* mycosis in the grasshopper *M. sanguinipes* compared to grasshoppers held in shaded conditions.

These results support the hypothesis that environmental conditions in the field may limit the effectiveness of *B. bassiana* as a biocontrol agent against orthopterans. Inglis et al. (1997b) documented this common problem. Field applications of *B. bassiana* on grasshoppers did not reduce populations under hot conditions, but mycosis occurred quickly after the grasshoppers were caged at cooler temperatures. The use of *B. bassiana* against rangeland insects whose normal or febrile T_{set} is above 35°C may only be practical when weather conditions do not allow thermoregulation.

EXPERIMENT #4: FIELD ENCLOSURE BEHAVIORAL OBSERVATIONS

Introduction

Concluding that Mormon crickets exhibit behavioral fever solely from thermal gradient observations may be premature if an elevation in T_b is not also observed in the field (Heinrich 1993). An insect's expression of a behavioral fever requires reacting to the pathogen by elevating T_b above its normal T_{set} , but it also requires changes in thermoregulation to maintain the febrile T_b . If Mormon crickets can exhibit a behavioral fever under field conditions when inoculated with *B. bassiana*, then inoculated crickets should shift their behavior in ways that would result in an increased T_b .

Groups of control and inoculated crickets held under similar field conditions should exhibit different proportions of thermoregulatory behaviors if Mormon crickets are expressing a behavioral fever. To raise their T_b , inoculated Mormon crickets should be in the basking posture more and spend more of their time in the full sun than control crickets. I should also find a lower proportion of inoculated crickets in the full shade.

Materials and Methods

I constructed eight additional cages at the same site and in the same manner as the cages used in Experiment #3 except that none were shaded. The two trials of this experiment ran concurrently with and used the same Mormon cricket stock as used in Trials #1 and #2 of Experiment #3. There were two replicates ($N = 15$ in each) of four treatments: 1) control female, 2) inoculated female, 3) control male, and 4) inoculated male. I randomized the placement of replicates in the eight cages in both trials.

At 30 min intervals from 0700 to 1700 I recorded on maps the position and posture of each cricket in each cage. On the maps I included the direction of the sun and the location of the shaded and lit areas in the cage. The postures Mormon crickets adopted in the cages differed little from the field observations. The perching, walking, basking, and stilting postures were present. Additionally, I observed a partial stilting posture where the Mormon crickets raised only one end of their body. In this posture crickets aligned their body parallel to the sun's rays with their head toward the sun. When they raised their anterior half and bent their head downward the pronotum flexed out from the body and shaded the top of the abdomen. I did not explicitly define or record a normal posture in the cage observations.

Allowing for acclimatization, I did not use observations in the analyses that were taken on the days crickets were initially placed in the enclosures in each trial. Clouds or rain interfered with the full range of thermoregulation capabilities on 8 of the 15 days of observation. I did not use observations from those days in the analyses because the pattern and frequency of thermoregulatory behaviors differed from that on warmer days. Crickets basked and remained in the sunlight until later in the day or returned to basking when cloud cover increased. On 3 of the 8 cloudy days T_s measurements were so low that it was unlikely that Mormon crickets could have reached T_{set} .

Plots of solar radiation measurements taken at the field site were smooth arcs on the remaining 5 days indicating cloudless or light cloud conditions. The maximum T_s in the cages was $> 51^\circ\text{C}$ on each of those 5 days. I collected T_s data in an unshaded cage used in Experiment #3 and used the maximum T_s value during each observation period to define six T_s categories.

I tallied cricket behavior from the maps by calculating the proportions of cricket behaviors and insolation levels for each cage at each time. I analyzed the behavior and insolation proportions using the same grouped χ^2 test as in Experiment #2. I did not

include the stiling posture in subsequent analyses because there were only 19 instances in the dataset.

Results

I took 9,144 observations on 246 different crickets during the 5 days included in the analysis. Over the course of the two trials there was 28.3% mortality in the control cages, 1.7% due to *B. bassiana* mycosis. In the inoculated cages, mortality was 37.3% and mycosis was 12.7%.

There were no differences in behaviors expressed or insolation choices between the control and inoculated groups. However the sexes exhibited different patterns of behavior, regardless of pathogen treatment. Males perched and basked more often than females (Fig. 14). A higher proportion of males were found in the full sun, but fewer were in the full shade (Fig. 15).

Several trends existed in the distribution of behaviors and insolation choices across T_s . The proportion of Mormon crickets perching increased as T_s increased (Fig. 16). The proportion of crickets in the partial stiling posture increased to a peak in the 40 - 44°C T_s category and then declined. Basking decreased as T_s increased. Mormon crickets chose full shade and partial shade more as T_s increased, and so were found less often in the full sun as T_s increased (Fig. 17).

Discussion

The evidence for a behavioral fever in *B. bassiana* inoculated Mormon crickets is poor. Although there was a slight increase in the T_b of inoculated crickets on the thermal gradient, without changes in thermoregulatory behavior the crickets cannot exhibit a behavioral fever under field conditions. The inoculated Mormon crickets in this

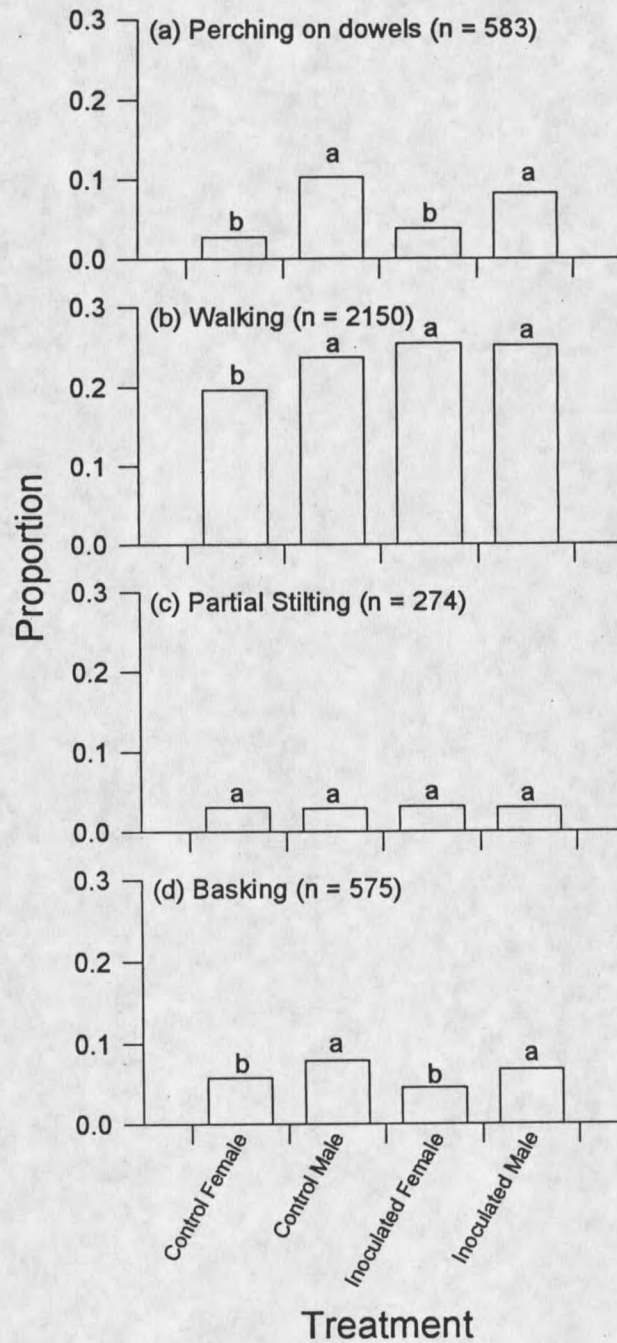


Figure 13. Proportion of Mormon cricket behaviors exhibited in different treatments. Means with different letters are different at $P < 0.05$ (grouped χ^2 test).

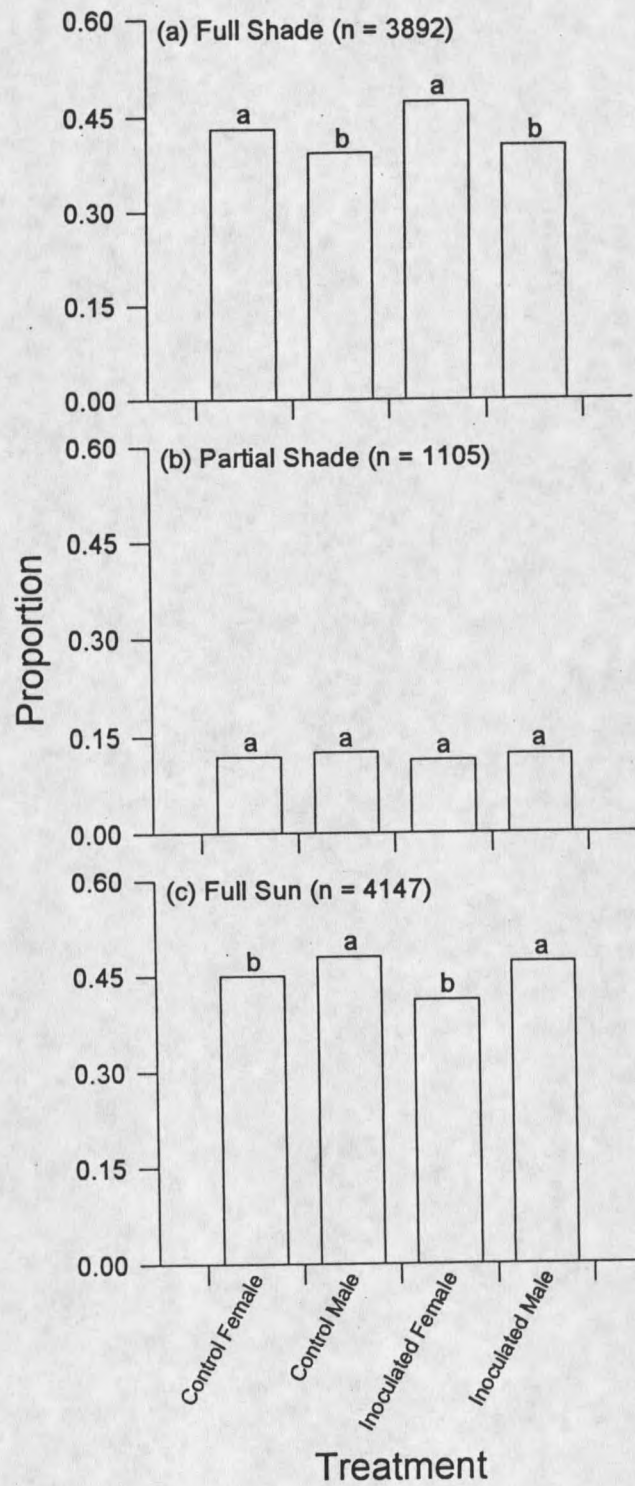


Figure 14. Proportion of Mormon cricket insolation choices in different treatments. Means with different letters are different at $P < 0.05$ (grouped χ^2 test).

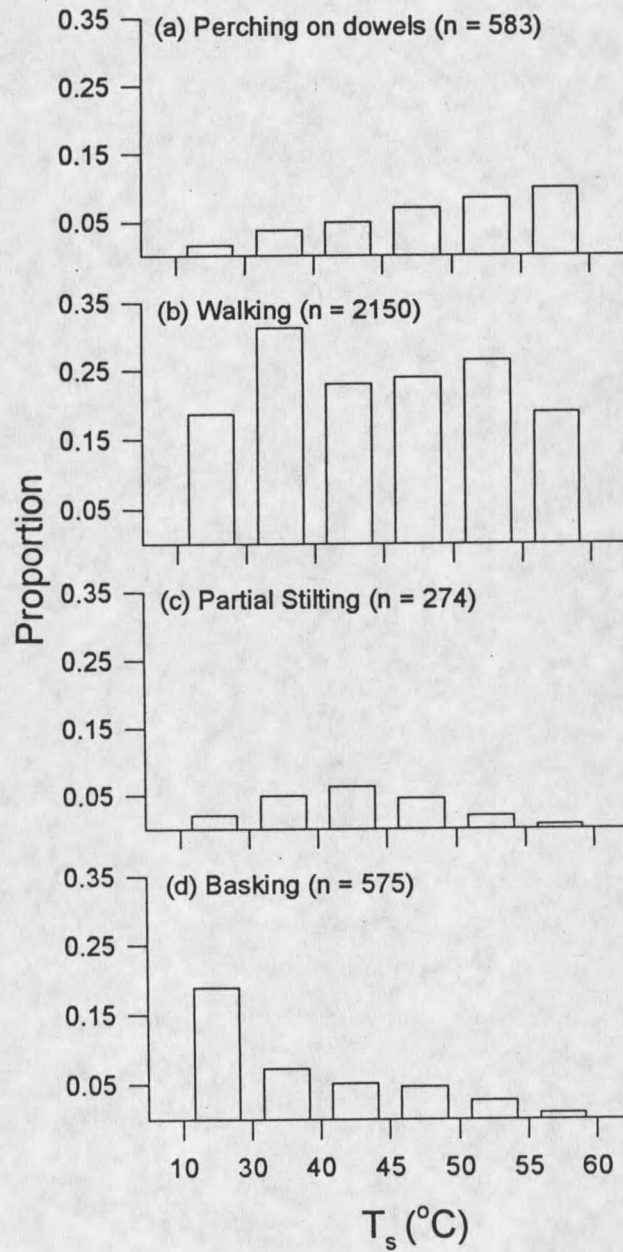


Figure 15. Proportion of Mormon cricket behaviors exhibited across T_s categories.

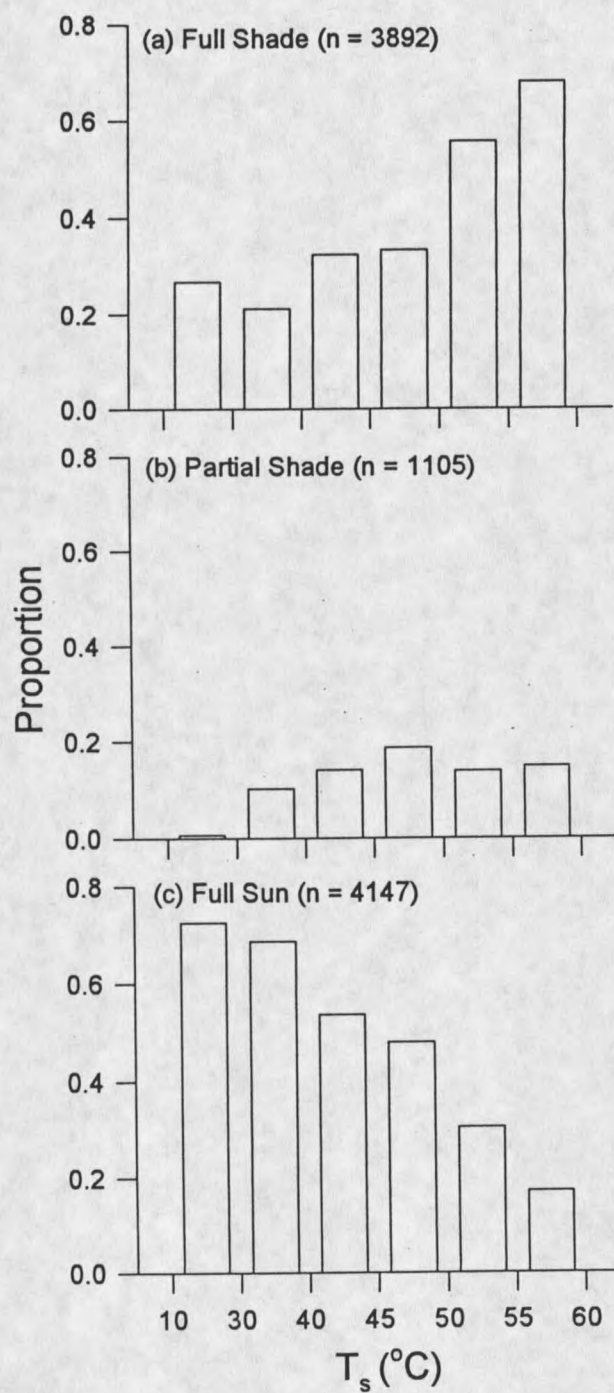


Figure 16. Proportion of Mormon cricket insolation choices across T_s categories.

experiment did not exhibit any behavioral differences that might lead to higher T_b 's than the controls.

Male Mormon crickets spent more time basking and exposed to the full sun, and less time in the full shade than females. However, I cannot conclude that the behavioral differences resulted in a higher T_b for males. Kemp (1986) found that the differently sized sexes of some grasshopper species increased their T_b at different rates, but the sexes controlled their T_b similarly. Mormon cricket males are smaller than the females (Gwynne 1984), and the difference in body sizes may affect posture or movement (Pepper and Hastings 1952) without necessarily leading to different T_{set} 's.

Mormon crickets that perched in shrubs when T_s was high in the field had lower mean T_b 's in the field samples, and the same may be true for those perched on dowels in the cages. The proportion of Mormon crickets perching on dowels increased as T_s increased, presumably as crickets sought a refuge from high soil temperatures (Fig. 16). However, the male bias in the proportion of crickets perching on dowels may be related to reproductive behavior not thermoregulation. Male Mormon crickets frequently called for mates while perched on the dowels. The observations of Gwynne (1984, 1993) confirm that males often call while perched in shrubs in natural populations.

Mormon crickets responded to low T_s by basking and high T_s by perching. At intermediate temperatures, the use of the partial stiling posture increased. Crickets that partially stilted remained on the ground and decreased their rate of heat gain by turning into the sun and shading their abdomens. With only a small change in body orientation and posture compared to basking, partial stiling allowed crickets to remain stationary and maintain a stable T_b while T_s increased.

In conclusion, Mormon crickets do not respond to *B. bassiana* inoculation with a behavioral fever. The small increase in mean T_b of inoculated crickets on the thermal gradient was not accompanied by a change in thermoregulatory behavior. However, the

prevalence of mycosis in Mormon cricket adults thermoregulating in natural conditions was less than in crickets restricted to lower temperatures. Without using a behavioral fever response, Mormon crickets successfully defended against *B. bassiana* colonization. Therefore the Mormon cricket's normal T_{set} , as found on the gradient and verified in the field, was high enough to limit fungal growth.

Mormon crickets could have maintained their T_{set} of approximately 36°C for several hours a day in the field cages. Maximum T_s values were greater than 37°C for an average of 5.2 hours per day during Trials #1 and #2 of the field cage mortality experiment. Mormon crickets inoculated with *B. bassiana* resisted mycosis when exposed to 37°C, but not 34°C, for 4 hours per day (O'Neill et al. unpublished data). Nymphs of *Melanophus sanguinipes* inoculated with the same strain of *B. bassiana* used here resisted mycosis when exposed to 40°C for 4 or more hours per day (Inglis et al. 1996). But nymphs held in 35°C incubation required continuous exposure to achieve a similar reduction in mycosis levels. *Beauveria bassiana* colonization in Mormon crickets is stopped by exposure to the crickets' normal T_{set} for 5 or more hours per day.

Carruthers et al. (1992) found a relationship similar to that for the Mormon cricket and *B. bassiana* between the clearwinged grasshopper, *Camnula pellucida*, and the fungus *Entomophaga grylli*. The grasshopper's normal T_{set} , as measured in the field, was 38 - 40°C. Incubation of inoculated crickets at 40°C for 2 or more hours per day significantly reduced fungal mycosis. A behavioral fever was not found.

Blanford et al. (1997) used field T_b sampling to detect a behavioral fever in the Senegalese grasshopper when sprayed with the fungus *Metarhizium flavoviride*. Treated populations raised their T_b 4 - 5°C above that of controls during the first five days after application. This research clearly showed a behavioral fever in a field situation. The small behavioral fever I found in Mormon crickets would be difficult to detect with field T_b sampling.

However, *B. bassiana* proved effective in a field application where host body temperature was limited by cool and cloudy weather conditions. Johnson and Goettel (1993) showed in field plots that *B. bassiana* reduced grasshopper populations by 60% nine days after application of dry conidia in a wheat bran carrier. During the trial, the daily maximum ambient temperatures were 16 - 22°C and daytime cloud cover was 20 - 60%. It is unlikely that grasshoppers in the field trial could have thermoregulated at their normal body temperatures and so might have been more susceptible to fungal mycosis.

Though *Beauveria* did not control pest populations in the field experiments reported here, the fungus may be effective when applied to take advantage of cooler environmental conditions that restrict host thermoregulation. *Beauveria bassiana* may significantly reduce Mormon cricket populations if field applications occur in the spring when temperatures are generally cooler and more variable. The early detection of possible outbreak populations then becomes critical if *B. bassiana* is used to control Mormon crickets.

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