Thermal Behavior of Two Acridid Species: Effects of Habitat and Season on Body Temperature and the Potential Impact on Biocontrol with Pathogens

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ABSTRACT Thermoregulatory behavior was studied in two key acridid pest species from west and south Africa. *Locustana pardalina* Walker (Orthoptera: Acrididae) from the arid Karoo region of South Africa was an active behavioral thermoregulator using postural adjustments and microhabitat selection to elevate and then maintain body temperatures at a preferred level between 38 and 41°C for much of the day. Both cool weather and time of season significantly affected the ability of these locusts to reach and maintain these preferred temperatures. *Hieroglyphus daganensis* Krauss (Orthoptera: Acrididae) from the humid tropical river valleys of west Africa was not an active behavioral thermoregulator and showed none of the postures or habitat selection associated with such behavior. Body temperatures varied little across the day being generally around 32°C. The humid, wet habitat where this species occurs and the uniformity of the thermal environment appeared to preclude the development of elaborate thermal behaviors seen in *L. pardalina* and other behaviorally thermoregulating ectotherms. The implications of host thermal behavior and the marked difference both within and between species under different environmental conditions are discussed in terms of biological control using a biopesticide based on an entomopathogenic fungus. It is concluded that the fundamental importance of host body temperature in relation to the fitness of the insect and its ability to cope with disease challenge, although generally overlooked in biocontrol programs, has significant implications for the successful development of microbial pest control.

KEY WORDS *Locustana pardalina*, *Hieroglyphus daganensis*, grasshoppers, thermal behavior, pathogens, biological control

BEHAVIORAL THERMOREGULATION BY ectothermic insects has been widely studied (May 1979, Heinrich 1981, Heinrich 1993). For acridids, the group containing the shorthorned grasshoppers and locusts, many of which are significant pests of commercial and subsistence agriculture, the elaboration and understanding of this gross thermal behavior has been studied for some time (Uvarov 1977 and references therein) and determinations of the preferred set point temperature, or at least the narrow range in which the insect prefers to operate, has been elucidated for a number of species (e.g., Waloff 1963, Chapman 1965, Stower and Griffiths 1966, Carruthers et al. 1992, Lactin and Johnson 1996, Willott 1997). The ability to regulate body temperature behaviorally and achieve preferred set points has fundamental consequences for activities such as feeding (Lactin and Johnson 1995), locomotion (Whitman 1988), reproduction (Begon 1983, Willott and Hassell 1998), habitat selection (Anderson et al. 1979, Gillis and Possai 1983), and in turn the rate of development (Begon 1983, Whitman 1988, Lactin et al. 1995). Overall, behavioral thermoregulation allows body temperatures to be attained that optimize physiological processes making the best use of enzyme systems and avoiding excess heat gain. Thus, in terms of physiological processes, insects that have achieved, and are maintaining, their preferred temperature can be described as being in their "healthiest" state.

One aspect generally overlooked, however, is the extent to which this fitness confers some degree of immunity to disease. Where this has been examined (Carruthers et al. 1992; Inglis et al. 1996; Blanford et al. 1998; Blanford et al. 1999a, 1999b) temperature and host thermal biology have been shown to have a significant impact on disease development and ultimate susceptibility of hosts to particular pathogens. The mechanisms involved appear to include both direct effects of temperature on the pathogen growth rate and survival, and the effects of preferred temperatures on the host immune response. Which of these is the most important and how their relative importance varies for different hosts, pathogens, and environments is unclear. What is clear, however, is that understanding host thermal behavior is crucial for predicting and interpreting (and even optimizing) the outcome of biocontrol using pathogens and for understanding the dynamics of insect pathogen interactions in general (Blanford and Thomas 1999b).

In this study we examine thermal behavior of two acridid species, *Locustana pardalina* Walker and

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Hieroglyphus daganensis Krauss. *L. pardalina* can be a serious pest across a large area of southern Africa and has been subject to almost uninterrupted control measures in its recession area (the Karoo region of South Africa) over the past 50 yr (Lomer et al. 1999). *H. daganensis* is an intermittent pest of rice in areas of clay-based grassland subject to seasonal flooding (Steedman 1990). This grasshopper is generally found in the major drainage basins of the Senegal, Niger, and Chad rivers. Both these species have been the target of biocontrol using a mycoinsecticide by the LUBILOSA program (Lomer et al. 1997, Price et al. 1997). This program has been developing a fungal entomopathogen (*Metarhizium anisopliae* variety *acridum*, formerly *Metarhizium flavoviride* Gams & Royzspal but now reclassified [Driver et al. 2000]) for control of grasshoppers and locusts in Africa. From previous laboratory results and field trials it is apparent that a considerable difference in the duration of disease incubation occurs among laboratory maintained samples, field cage maintained samples, and field sprayed populations (e.g., Langewald et al. 1997). It is also apparent that such findings cannot be extrapolated simply to all target acridids. For example, *L. pardalina* shows rapid mortality under laboratory conditions (Bateman et al. 1994), an increased incubation period in field cages, and a further increase in field enclosures (Price et al. 1997), whereas for *H. daganensis*, a comparable delay is not apparent (Lomer et al. 1997).

The aim of the current study was to examine how the thermal biology of these two target species differ in relation to the contrasting environments they occupy and to highlight the potential consequences of the observed behaviors for the efficacy of the biocontrol program based on the LUBILOSA biopesticide and the implications for other potential microbial biocontrol agents.

Materials and Methods

Locustana pardalina. Field work on *L. pardalina* was carried out during February 1998 near the town of Britstown, and May 1998 near the town of Van Wyksvlei in the Karoo district of South Africa.

During February, one band of immature adults was identified and the thermoregulatory behavior monitored periodically over 3 d during a period of cool temperatures and intermittent rain. Individual locusts were either hand caught or trapped in a small sweep net. Internal body temperatures were measured with fine wire (0.125 mm diameter) copper constantan thermocouples (Omega, Newport, UK) in conjunction with a hand-held digital thermometer (Omega) that gave rapid response readings and accuracy to 0.1°C. Thermocouple tips were inserted ≈2 mm into the thorax after a small hole had been made with the tip of a 0.22-mm-diameter hypodermic needle. Individual locusts were generally processed within 7–10 s of capture. Where processing took longer, insects were discarded and measurements not recorded. Environmental monitoring was conducted using Squirrel data loggers (Grant Instruments, Poole, UK). Temperature of soil surface in shade and sun, and the air temperature in a variety of positions from "insect height" (≈0.5–1 cm above soil surface) to canopy ceiling (0.5–0.75 m) were recorded with 14-mm-diameter thermisters attached to the data loggers. Solar radiation was measured with a silicon cell pyranometer (Skye Instruments, Llandrindod Wells, UK) and wind speed at 0.5 m with an anemometer (Skye Instruments). All environmental measurements were logged every minute during daylight hours and every 10 min during the night. General observations were made on the distribution of locusts and postures associated with behavioral thermoregulation during both day and night.

After the monitoring of the adult band above, ≈300 adults were collected and placed in a large field enclosure measuring 16 by 16 m. Enclosure walls were made of black plastic sheeting that was smooth enough to stop the locusts climbing out. Enclosure walls were 0.75 m high. Vegetation in the enclosure was left undisturbed apart from a 0.5-m barrier running along the inside of the enclosure walls where all vegetation was removed. The adults wings were cut ≈1 cm from the thorax. This effectively limited their flying ability such that they could not escape, but careful observation suggested that other behaviors, particularly thermoregulation, was unaffected. Bateman et al. (1998) had previously used paper clips to immobilize adult *Schistocerca gregaria* (Forskål) in arena trials. However, it was felt that the proximity of a metal paper clip to the insect body under potentially hot conditions may have affected thermal behavior. Body temperature, monitoring of environmental conditions, and observations of locust behavior were carried out as described above over 4 d. As continual monitoring may have increased the disturbance to locusts and affected their natural diurnal movement, and because numbers were limited, body temperatures were measured for short periods over all the days and then combined to provide an overall pattern of behavior.

During May 1998 a similar enclosure to the one described above (though slightly larger at 20 by 20 m) was constructed and ≈1,000 fourth- and fifth-instar hoppers were placed inside. These were monitored over a 6-d period. Body temperature measurements and recording of environmental conditions were carried out as above, as were observations of the diurnal and nocturnal pattern of the locust behavior.

Hieroglyphus daganensis. Studies on *H. daganensis* were performed in late July and early August 1996 in north Benin, West Africa. Field populations (third–fifth instar) were monitored, by using procedures described above, in or on the edge of reedy pools and rice paddies in the Niger river valley close to the town of Malanville.

Because field densities were low and capture quite difficult, in addition to the free moving insects, a number of grasshoppers were captured and tethered to thin bamboo canes placed at intervals in one of these pools. Each insect was tied via a slip noose (placed between the front pair of legs and circling the pronotal

Table 1. Environmental temperature and solar radiation parameters during monitoring in the Karoo in February and May 1998

Pop/Month		Soil surface 0700–1900 hours	Ambient temp 0700–1900 hours	Night temp 1900–0700 hours	Solar radiation (W/m^{-2}) 0700–1900 hours
Field population/Feb.	Mean ± SEM	23.8 ± 0.20	21.5 ± 0.11	17.8 ± 0.12	221.9 ± 4.65
	Max	44	36.6	25.3	720
	Min.	15.4	14	13.7	5
Enclosure population/Feb.	Mean ± SEM	41.4 ± 0.33	33.1 ± 0.074	18.6 ± 0.10	601.6 ± 15.19
	Max	54.8	39.6	25.2	1,200
	Min.	24.8	18.6	14.6	5
Enclosure population/May	Mean ± SEM	32.3 ± 0.55	24.2 ± 0.17	11.9 ± 0.078	458.0 ± 13.56
	Max	51	37.8	26	930
	Min.	5.4	4	2.2	5

shield) to a thin piece of cotton that was tied in turn to the cane (see Thomas et al. 1998). Insects had freedom of movement to the extent of the cotton, reaching from water surface to the top of the vegetation canopy. Body temperature of these insects was monitored once and then individuals were replaced. No readings were taken if the insect was entangled or in anyway showing signs of stress from the tether. Although this monitoring continued, regular samples were taken of the naturally occurring population in and around the pool. Insects were captured with a sweep net at a variety of sights from pool edge to reeds in the center and body temperature taken in the manner described above and related to environmental conditions at that time.

Environmental variables were recorded as above, although because of concerns over the security of the equipment at night, no nighttime data were collected.

Results

Locustana pardalina. Conditions during the monitoring of the adult band before the construction of the enclosure were cool and wet. Locusts remained on a small hill where they were initially found, throughout the 3 d of the monitoring period. Mean air temperature (pooled for a range of heights above ground in protected and exposed positions), soil surface temperature, night temperatures (pooled for soil surface and range of heights above ground), and solar radiation are given in Table 1.

As an example of the conditions, mean hourly body temperature and ambient temperature in a variety of positions for day 2 of the period are shown in Fig. 1. Overall, daytime mean ± SEM body temperatures were 25.9 ± 0.55°C, with maximum 40.6 and minimum 15.7°C. Cool conditions and the rainfall accompanying them were uncharacteristic for the time of year and the classical diurnal movement and posturing of thermoregulating locusts were only apparent during brief periods. Only during the afternoon of day 2 were locusts able to raise body temperature between ≈1400 and 1600 hours ([mean ± SEM] 37.9 ± 0.38°C, maximum 40.6°C, minimum 33.7°C, $n = 22$ [see Fig. 1]). Either side of this period heavy cloud cover limited body temperature to some 6–7°C lower (31.9 ± 0.23°C, $n = 34$). In addition, on day 2 of this period locusts did not vacate roosting sites until 0930 to 1000 hours. Soil surfaces, on the whole, remained wet and therefore the faster-drying exposed rocks were selected by the locusts in an attempt to elevate body temperatures, although maximum temperature achieved was only 26.4°C. Locusts vacated soil surfaces early in the afternoon for roosting sites at the onset of rains. Some adults aggregated into tight clumps around the main stem of the shrubs. When body temperatures were sampled at night (2200–2300 hours) they revealed a mean ± SEM of 21.6 ± 0.26°C, $n = 15$ in these aggregations. Freshly killed locusts placed in more exposed positions at the same time had mean body temperatures of 15.5 ± 0.17°C, $n = 4$. These dead locusts were fixed adjacent to individually roosting adults and as such were expected to mimic their body temperature closely. Air temperature during this period ranged between 14.4 and 19.1°C. By morning, all locusts, whether in these aggregations or not, had similar body temperatures of between 15.7 and 17.7°C.

Monitoring locusts in the field enclosure after this period of cool weather revealed a very different pattern of thermal behavior as ambient temperatures increased. Conditions during this monitoring period

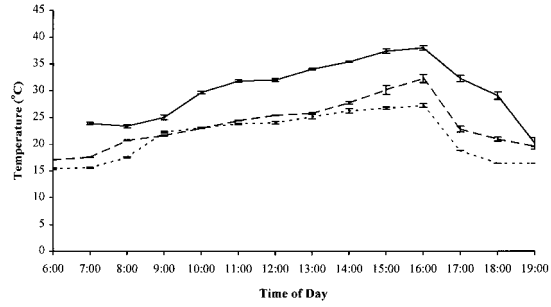

Fig. 1. Daily profile of *L. pardalina* body temperature and ambient temperature on day 2 of the first field study in February 1998. Mean ± SEM temperatures were estimated from measurements recorded between 0700 and 1900 hours. Mean body temperature is represented by the solid line; soil surface by the dashed line and air temperature by the dotted line. Ambient air temperatures are means pooled from data logger readings taken at heights from 0. 5 cm to 0.5 m in both sun and shade. Soil surface temperatures represent means from recordings taken from exposed positions. Each hourly mean for body temperature represents a minimum of 12 readings.

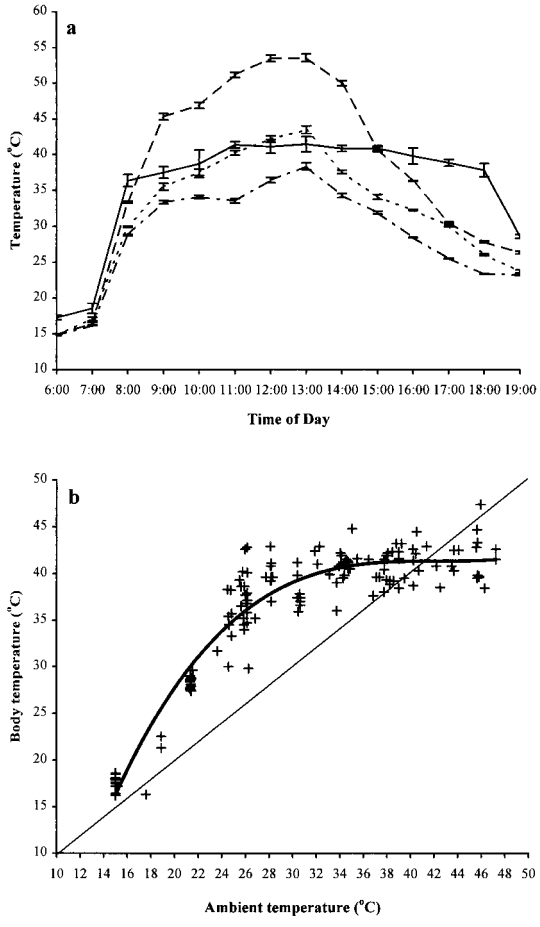

Fig. 2. Pattern of thermoregulation for the *L. pardalina* from the February enclosure group. (a) Profile of mean hourly body temperature (solid line) with exposed soil surface temperature (dashed line), air temperature at "insect height" on the soil (0.5 cm) (dotted line) and air temperature at a variety of heights (0.2–0.5 m) in both sun and shade (dotted and dashed line). Hourly means represent a minimum of 14 recordings. (b) Distribution of body temperature (Tb) against ambient temperature (Ta) for this group. Straight line shows a null model for no active thermoregulation whereby Tb = Ta. The curve shows best fit nonlinear regression describing the pattern of active thermoregulation. Regression equation is $Tb = 0.0012Ta^3 - 0.1574Ta^2 + 6.6395Ta - 52.089$. $R^2 = 0.90$, $P < 0.001$, $n = 167$.

were characteristically hot, with no rainfall events. Mean hourly body temperature (pooled for the 5-d monitoring) is shown in Fig. 2a together with hourly means for ambient temperature from a variety of positions. Mean daytime air temperature (pooled as above), soil surface temperature, night temperature (pooled as above), and solar radiation are given in Table 1. Overall, body temperature of adults measured in the field over the first 3 d of cool weather (above) were significantly lower than those measured in the enclosure during the hotter weather ($t = 12.22$, df = 288, $P < 0.001$). For the enclosure group, where optimal thermoregulation was possible, the flattening of the daily profile between 0800 and 1800 hours represents the preferred temperature of *L. pardalina.* Mean body temperature (±SEM) during this period was 39.6 ± 0.28°C, with a maximum of 47.4°C and minimum 33.3°C. These results also indicate that during normal conditions of midsummer, *L. pardalina* can spend some 10 h of the day with body temperatures >35°C, with 8 h close to 40°C.

Locusts maintained internal body temperatures in excess of 35°C until ≈18:20, whereupon they fell gradually throughout the night (2000 hours mean ± SEM body temperature 26.1 ± 0.49°C, $n = 22$; 2300 hours 24.4 ± 0.15°C, $n = 25$; 0400 hours 15.4 ± 0.19°C, $n = 19$). Body temperature and the pattern of cooling tracked air temperature closely, which was only 1–2°C in excess of air temperature at the insects position. No aggregations of locusts were observed in roosting positions as had been noted for the three days monitoring of the field population above.

Nonlinear regression of body temperature on ambient temperature (Fig. 2b) illustrates the rapid elevation in body temperatures as ambient temperatures increased (via basking on soil surfaces) followed by body temperatures reaching an equilibrium across a range of environmental temperatures (stilting above soil surface followed by ascent into vegetation).

For the third monitoring period, during May 1998, because sample sizes were low, mean hourly body temperature was pooled over three 2-d periods to provide sufficient data points to cover the whole daylight period (Fig. 3). Ambient environmental temperatures and solar radiation for this period are summarized in Tables 2 and 3. The pattern of temperature fluctuation across study days is shown in Fig. 4. Overall, locusts maintained mean daytime body temperatures ≈5°C lower than those in the February enclosure study with a significant difference between periods ($t = 7.13$, df = 212, $P < 0.001$). Mean body temperature during the hottest part of the day (1000–1500 hours) pooled for all days monitored was 36.5 ± 0.16°C, with maximum 44.3°C and minimum 24.5°C. Ambient temperatures experienced by the locusts in May were cooler than those in February. Only on day 1 were characteristic heat avoidance behaviors observed (midday ascent). After day 1, temperatures just above soil surface never reached high enough levels to necessitate ascent into vegetation and elaborate heat avoidance behaviors. Temperature fell on days 2 and 3 before rising again on days 4, 5, and 6 (Table 2; Fig. 3). However, the magnitude and duration of the hottest temperatures on the latter days were not as great as those recorded on the first day. Nighttime temperatures were also lower than in February (see Tables 1 and 2) dropping to 2.2–10.4°C in the early morning. On day 1, body temperatures between 1000 and 1500 hours averaged 38.2 ± 0.18°C maximum 44.3°C, minimum 32.1°C and combining days 2–6, mean body temperature was 35.6 ± 0.15°C, with maximum 41.2°C and minimum 24.5°C. The change in climatic conditions during the 6-d monitoring can be used to explain the change in behavior and spatial distribution of the

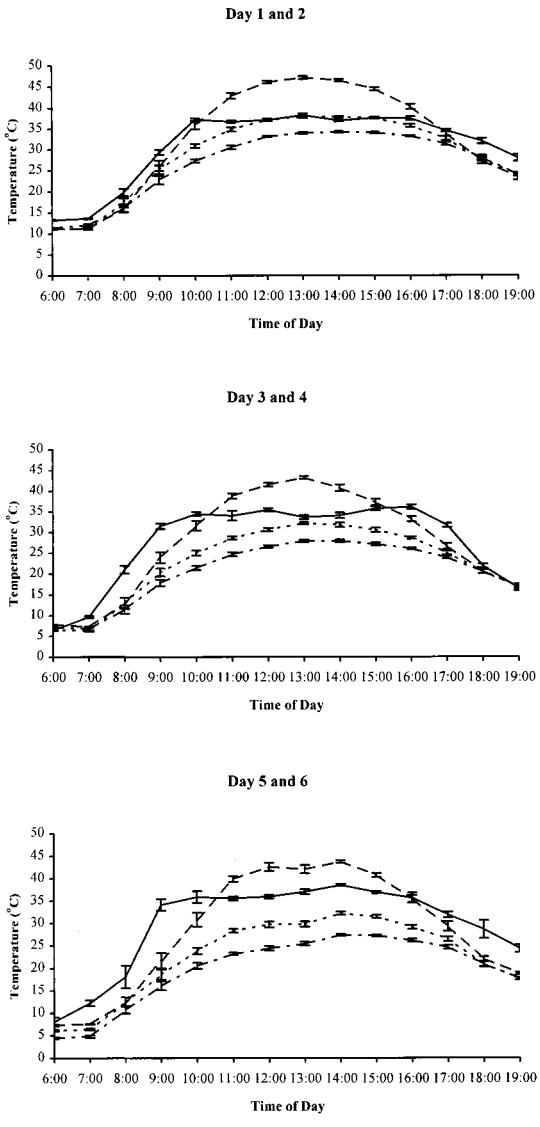

Fig. 3. Mean ± SEM profiles of *L. pardalina* body temperature in May. Mean hourly body temperatures (±SEM) for 2-d combinations (solid line); soil surface temperature (dashed line); air temperature at insect height above soil (dotted line), and air temperature at 0.4 m (canopy ceiling) (dotted and dashed line). Body temperatures represent a minimum of 19 readings.

locusts within the enclosure. During day 1, soil surface temperatures were >40°C for 6 h between 0900 and 1600 hours (44.0 ± 1.03°C) and >39°C for 3 h 0.5 cm above soil surface. Accordingly, locust behavior on this day showed the normal pattern of movement including ascent for heat avoidance. Mean soil surface temperatures during days 2–6 were cooler than day 1 (see Table 2) and temperatures on the soil >40°C only lasted for 6, 1, 4, 4, and 4 h on these days, respectively. Temperatures at insect height never reached 39°C on these days. Ground basking behavior could be observed throughout the day, on all these days, and many locusts remained in basking groups for long periods. No heat avoidance behaviors (shade seeking, movement to off ground sites) were observed.

During this period in May, some unusual aggregative behavior was noted. Clumping of locusts into mounds (particularly on day 1) resulted in body temperatures during the evening and night being in excess of locust that had ascended bushes for roosting. Over the six study days, mean body temperature of locusts sampled from these mounds (between 1800 hours and midnight) was 27.8 ± 0.79°C (maximum 37.8°C, minimum 19.9°C), whereas mean body temperature of individually roosting locusts was 21.1 ± 0.59°C (maximum 30.9°C, minimum 13.8°C) over the same period. This difference was significant ($t = 6.88$, df = 108, $P = 0.001$) and shows that where mound-forming behaviors occur body temperatures can be maintained at a higher level for a longer period than individually roosting hoppers.

Comparing mean body temperature profiles of the three study periods (Fig. 4) indicates the effect of within season fluctuations of temperature and solar radiation caused by rain events (February field population), the distribution and level of body temperatures under optimal thermoregulatory conditions (February enclosure population), and the limitations to achieving preferred body temperatures at the end of the season (May enclosure group).

Hieroglyphus daganensis. Mean body temperature and mean hourly ambient temperature (pooled for a range of heights between water surface and canopy ceiling) are shown in Fig. 5a. Direct observation revealed that *H. daganensis* did not exhibit any of the postures classically associated with thermoregulating grasshoppers. Nor did they appear to show any particular diurnal movement in the thermal gradient from the cooler water surface (28°C) to the hotter top of the canopy (34–38.6°C during one brief period). Mean body temperature for *H. daganensis* over the study period was 31.8 ± 0.21°C (maximum 39°C, minimum 25.1°C). Ambient temperature over the same period was 28.8 ± 0.06°C, (maximum 38.6°C, minimum 25.6°C). Mean range of both body temperature and ambient temperature was small being 6.5 and 5.1°C,

Table 2. Environmental temperature and solar radiation parameters (mean ± SEM) during the six study days in May 1998 of *L. paradalina*

Parameters	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6
Soil surface	44.0 ± 1.03	41.2 ± 0.67	36.4 ± 0.60	39.0 ± 1.02	38.5 ± 1.05	38.9 ± 0.86
Ambient temp	33.6 ± 0.56	29.3 ± 1.73	23.6 ± 1.32	24.6 ± 1.94	23.5 ± 1.79	23.2 ± 1.40
Night temp	14.8 ± 1.03	14.3 ± 1.32	9.3 ± 0.89	10.5 ± 1.27	12.1 ± 0.83	14.0 ± 0.80

Table 3. Solar radiation (Wm^{-2}) values for the six study days in May 1998 for *L. paradalina*

	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6
Mean ± SEM	480.1 ± 33.59	395.3 ± 33.52	406.9 ± 34.24	388.8 ± 35.22	388.5 ± 34.50	350.0 ± 31.07
Max	815	795	810	930	800	880

respectively. Relative humidity ranged between 51.9% at the top of the canopy to 95.7% during the early morning and evening regardless of site in the canopy. Mean relative humidity was 79.8 ± 0.52%. Mean solar radiation (Watts m^{-2}) was 312.5 ± 7.90 W m^{-2} (maximum 1,220 W m^{-2}, minimum 35 W m^{-2}). Linear regression of ambient temperature on body temperature is shown in Fig. 5b. The slope of the regression line is not significantly different to one (slope = 0.9827, $t = 0.29$, df = 39, $P > 0.5$) and indicates that as ambient temperature increases there is a corresponding rise in body temperature.

Discussion

Thermal Biology. Use of regression analysis has been criticized previously when it has been used singularly to demonstrate active behavioral thermoregulation (e.g., Dreisig 1984, Hertz et al. 1993). However, this study was not designed specifically to describe the physiological ecology of the study species but to demonstrate the level of elevation of body temperature and the degree to which this can be maintained under varying climactic and seasonal conditions and species/habitat differences. To this end, combinations of regression analysis, profiles of mean hourly body temperature, and descriptions of thermally mediated behavior have been employed and comment has been made on the likely behavioral category (i.e., thermoregulator or thermal conformer) that these two species fall into.

The degree to which *L. pardalina* maintains body temperature at a steady level (Fig. 2b) during midseason suggests that this species is an active behavioral thermoregulator. Its preferred temperature, determined from the February enclosure population where environmental conditions permitted the full range of thermoregulatory postures and microhabitat selection, was around 39–41°C. This is close to the preferred temperature determined by Smit (1960) and

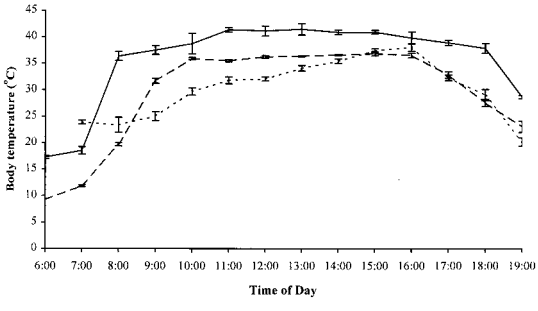

Fig. 4. Combination of mean hourly profiles (±SEM) for *L. pardalina* from February and May illustrating differing effects of cool weather pattern, optimal thermal conditions, and seasonal constraints. February field group from Fig. 1 (dotted line); February enclosure group from Fig. 2a (solid line) and pooled mean hourly body temperature for the 6-d period in May (dashed line).

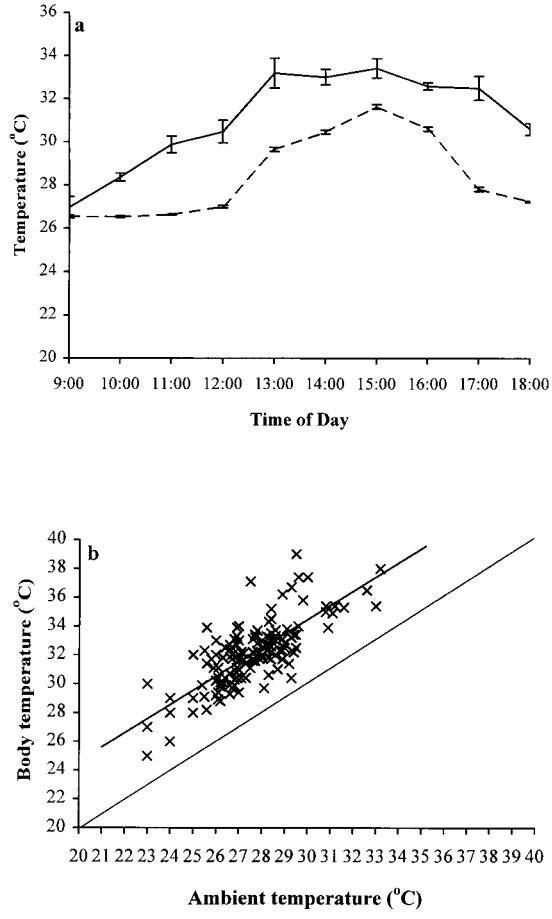

Fig. 5. *H. daganensis* body temperature and ambient temperature recorded in north Benin in July/August 1996. (a) Mean ± SEM hourly body temperatures (solid line) are estimated from recordings made over 4 d. Ambient temperature (dashed line) represents pooled recordings from data loggers at three different heights in the vegetation canopy. Each hourly body temperature represents a minimum of 16 readings. (b) Distribution of body temperature (Tb) against ambient temperature (Ta) for *Hieroglyphus daganensis* recorded over 5 d. The straight line shows a null model for no active thermoregulation, whereby Tb = Ta. The curve shows best fit linear regression describing the pattern of body temperature for *H. daganensis.* Regression equation is Tb = 0.9872Ta + 4.978. $R^2 = 0.64$, $n = 157$.

within the range of preferred temperatures reported for other locust and grasshopper species inhabiting arid or seasonally constrained environments, e.g., see Chapman (1965) for *Schistocerca gregaria* (Forskål), Lactin and Johnson (1996) for *Melanoplus sanguinipes* F., Carruthers et al. (1992) for *Camnula pellucida* Scudder, and Blanford et al. (1998) for *Oedaleus senegalensis* Krauss. Although the profile curve during May also appears to show some equilibrium body temperature level, it is clear that this is the maximal temperature that can be achieved because locusts were continually having to adopt heat gaining behaviors (basking on soil surfaces) to maintain this level, and environmental temperatures were not as high as those in February.

From the first study in February, it is apparent that short-term changes in the thermal environment during otherwise optimal thermoregulatory conditions can severely impact on the ability of *L. pardalina* to reach and maintain its preferred body temperature. Under such conditions the range of physiological, biochemical, and behavioral activities are likely to operate suboptimally (Heinrich 1977). Persistence of cool weather patterns or frequent reoccurrence have fundamental implications for the locusts ability to complete development and reproduce (Begon 1983, Whitman 1988) and, in the context of this study, to combat infections, as discussed below.

Toward the end of the season in May, locusts were found to be unable to elevate body temperatures consistently to their preferred set point. The preferred temperature was reached later in the day and then fell earlier in the evening than the group sampled in the enclosure in February (Fig. 4). These thermal constraints apparently led to the development of complex aggregative thermal behaviors resulting in body temperature being maintained longer than locusts acting individually. Similar aggregative behavior was also apparent in the February field population during the coolest period with the aggregation of the adults into roosting "clumps" at the base of the main stems of shrubs serving a similar purpose to the mounds formed on the ground in May by fourth- and fifth-instar hoppers.

From the evidence of the data collected on *H. daganensis*, this grasshopper does not seem to be an active thermoregulator. Unlike *L. pardalina*, behaviors such as basking, flanking, and stilting were not apparent, nor did *H. daganensis* make use of sparsely vegetated soil surfaces at the perimeter of the pools. The use of linear regression to describe body temperature in relation to ambient is valid in this case because the range of ambient temperatures is small. The fact that the slope of the regression does not differ from unity supports the lack of directly observable thermally mediated postures. Although body temperature is different to ambient this may be purely the result of the passive absorption of solar radiation and temperature by a relatively large bodied insect and as such this insect is likely to be more of a thermal generalist (i.e., one that moves through a mosaic of temperatures rather than a thermal conformer remaining sedentary and accepting fluctuations in ambient temperature).

Studies dealing with acridids in uniform thermal environments are few. However, the report of Anderson et al. (1979) concerning a grasshopper inhabiting a moist environment has direct parallels with *H. daganensis* in this study. In addition, the position of *H. daganensis* in the canopy may be considerably influenced by predation. Thomas et al. (1998) showed high levels of predation of *H. daganensis*, much of which was caused by frogs and toads. Thus, it can be inferred that grasshoppers choosing perching sites close to the water surface are likely to be predated. Grasshoppers choosing perching sites at the top of the canopy potentially also expose themselves to predation or excessive temperatures. Perching sites in midcanopy provide a refuge from both of these pressures but necessarily limit the range of environmental temperatures that the grasshopper can experience.

Implications for Biocontrol. Such variety in thermal behavior, both within and between species under different environmental conditions, has important implications for locust and grasshopper fitness and the likely impact of microbial pathogens.

Carruthers et al. (1992) showed that body temperatures in excess of 35°C for 4 h per day severely limit the development of, and can even eliminate, *Entomophaga grylli* Fresenius from host hemocoel. Inglis et al. (1996) have also shown that mortality from infection with *Beauveria bassiana* (Balsamo) Vuillemin is reduced to zero under similar conditions (or at least under conditions where optimal host thermoregulation is allowed). Thus, *L. pardalina* thermoregulating optimally at 35–41°C for up to 10 h per day would be expected to be effectively immune to these diseases.

Metarhizium anisopliae variety *acridum* has a higher thermal tolerance than the pathogens mentioned above (developing most rapidly at ≈30°C and not stopping growth until 40°C [Thomas and Jenkins 1997]). Pathogen growth would be expected to be severely limited under optimal thermoregulatory conditions. This is especially so if one considers that certain acridids infected with *M. anisopliae* variety *acridum* have been shown to develop a behavioral fever that increases the preferred body temperature by some 2–3°C (Blanford et al. 1998). Thus, it would appear that thermoregulatory behavior under optimal conditions is likely to hamper development of a range of pathogens, and particularly those whose thermal limits fall below that at which the locust regulates. However, this simple description masks many important biological details. For example, where *E. grylli* can be eliminated by host thermoregulation, *B. bassiana* and *M. anisopliae* variety *acridum* appear able to persist within the host even under optimal thermoregulation (Blanford and Thomas 1999b). Consequently, should environmental conditions change and the thermal constraint be lifted, these pathogens can recover and effect significant mortality. This mortality rate is influenced by diurnal fluctuations and the extent to which nighttime temperatures (when thermoregulation is not possible) are permissive for growth. This

applies particularly to *M. anisopliae* variety *acridum*, which does appear to grow within the host as soon as appropriate "windows of opportunity" present themselves (Blanford et al. 1998). For *B. bassiana*, however, high daytime temperatures appear to have a knock-on effect during the night and limit pathogen induced mortality even when nighttime temperatures appear conducive for growth (Inglis et al. 1996). If daytime temperatures fall, however, *B. bassiana* can recommence growth and cause mortality (Blanford and Thomas 1999b). Thus, even the relationship between mortality and nighttime temperatures is not straightforward. Moreover, some of the behavioral changes associated with suboptimal thermal conditions (such as aggregative basking and generally lower movement and dispersal) are likely to influence contact rate between individuals and the potential for horizontal disease transmission, thus affecting impact of any pathogen still further.

Empirical support for effects of temperature on pathogen efficacy can be derived from results of field trials of *M. anisopliae* variety *acridum* against *L. pardalina* conducted by the LUBILOSA program. A range of tests have been carried out in both the laboratory and field. Laboratory bioassays and studies using insects sprayed in the field and transferred to the laboratory and maintained under constant thermal conditions show the pathogen to be virulent against *L. pardalina*, causing 80–100% mortality (depending on the efficacy of the application) in 7–14 d (Bateman et al. 1994). Studies using insects maintained in field enclosures, however, show much more variable results. One study conducted on fifth-instar hoppers showed quite rapid control with up to 98% mortality after 21 d for 10 treated hopper bands (Price et al. 1997). A recent study on adults, in contrast, showed that although insects transferred to the laboratory died characteristically quickly, those maintained in field enclosures (actually throughout the warm February and March period of the current study) died much more slowly with 80% mortality only achieved after 70 d (Arthurs and Thomas 2000). This mortality was still significantly greater than controls, however, and treated adults also showed significant reduction in their weight, size of fat bodies (important for reproduction, flight, and dispersal capability), and number of eggs laid per pod (Arthurs and Thomas 2000), indicating that the pathogen still had an effect, albeit much more slowly. Given that thermal requirements of late instar hoppers and adults are likely to be the same (Chapman 1965, Lactin and Johnson 1995) then the dramatic difference between these field studies, and certainly between the laboratory and field results, indicates a major influence of environmental conditions (and hence thermal behavior) on pathogen performance, with considerable variation over time.

In contrast to *L. pardalina*, *H. daganensis* would appear unlikely to influence development of any diseases through processes of active thermoregulation and may, perhaps, be particularly vulnerable to a range of pathogens. This is confirmed by results of field trials using the LUBILOSA biopesticide, which show no difference in mortality rates between insects maintained under uniform conditions in the laboratory after spraying and insects left under natural conditions in the field (Thomas et al. 1997).

It can be suggested, however, that the habitat itself is likely to influence the exposure of hosts to pathogens. That is, soils act as a reservoir for entomopathogens, and recycling from this reservoir to infect insects on foliage is a key factor in pathogen-persistence and host-pathogen dynamics (Hochberg 1989). Inglis et al. (1993) have shown that *B. bassiana* is rapidly washed down from vegetation surfaces into this soil reservoir. In the *H. daganensis* system, however, the "soil surface" is often water (as is the case in this study), which may act as a sink, effectively removing pathogen load from the environment and minimizing chances of infection. Protection from microbial infection in this system, therefore, may have little to do with thermal behavior and more to do with the type of habitat occupied.

From the discussion above it can be seen that thermal behavior and the environmental factors behind the behaviors are variable and complex. It can also be seen that the potential implications for host-pathogen dynamics and the effectiveness of biocontrol using pathogens are equally complex. Nonetheless, the effects of thermal biology on host pathogen interactions have been largely overlooked in the majority of both fundamental and applied studies to date (Blanford and Thomas 1999a, 1999b). For the particular species discussed here, it is clear that the harsh environment and the active thermoregulation of *L. pardalina* represent a severe challenge for pathogen growth and undoubtedly contribute to the variability in performance of the LUBILOSA biopesticide. The need to account for thermal behavior and within and between season changes in environmental conditions will be essential for the effective implementation of this biopesticide in future *L. pardalina* control programs. For *H. daganensis*, it appears that the constraints may be less and potential use of the biopesticide easier although if this is the case, then it will be due more to a fortuitous combination of pathogen, habitat, and host behavior than any fundamental insight a priori into the host–habitat interaction.

Overall, it is clear that pathogens cannot simply be sprayed into the environment and be expected to perform in a similar, and reliable, manner to the chemical pesticides they are meant to replace. Appropriate use strategies need to be built around a comprehensive understanding of the biology of host, pathogen, and their interaction. The lack of such understanding has undoubtedly contributed to the notoriously patchy performance of microbial agents and biopesticides in many previous studies (Lisansky 1997).

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