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Thermal ecology of *Zonocerus variegatus* and its effects on biocontrol using pathogens

Simon Blanford, Matthew B. Thomas and Jürgen Langewald*

*Leverhulme Unit for Population Biology and Biological Control, NERC Centre for Population Biology and CABI Bioscience, Imperial College, Silwood Park, Ascot, Berks, SL5 7PY, U.K. and *IITA Plant Health Management Division Biological Control Centre for Africa, Cotonou, Benin*

Abstract

1 Thermal behaviour of the variegated grasshopper, *Zonocerus variegatus*, was investigated in the humid tropical zone of southern Benin, west Africa, in the dry seasons of 1996 and 1998. In 1998, investigations included studies of a population of grasshoppers sprayed with an oil-based formulation of the entomopathogenic fungus *Metarhizium anisopliae* var *acridum*.

2 Body temperature measurements and observations of thermal behaviour both in the field and on thermal gradients in the laboratory, suggest that *Z. variegatus* was not an active behavioural thermoregulator. Although it did show shade-seeking behaviour at high temperatures, no overt behavioural postures or microhabitat selection associated with heat gain and elevation of body temperatures was observed. Moreover, no alterations to thermal behaviour were found in response to infection by *Metarhizium*.

3 Body temperatures exhibited by *Z. variegatus* in the field will lengthen disease incubation of *M. anisopliae* var *acridum* compared with laboratory maintained, constant temperature conditions and may have a significant impact on pathogens with a lower thermal tolerance.

4 Habitat structure appeared to be an important factor determining the extent of body temperature elevation. The effect of habitat differences on infection and growth of *M. anisopliae* var *acridum* and other entomopathogenic fungi is discussed.

Keywords Biological control, fungal pathogens, grasshoppers, *Metarhizium anisopliae* var *acridum*, temperature, thermal behaviour, *Zonocerus variegatus.*

Introduction

Recently some studies have highlighted the therapeutic role that thermoregulation has for locust and grasshopper hosts in response to infection by a range of fungal pathogens (Carruthers *et al*., 1992; Inglis *et al*., 1996; Blanford & Thomas, 1999). This behaviour has a profound role not only in shaping the population dynamics of these species [and by implication the dynamics of other thermoregulating insects (e.g. Begon, 1983)], but in determining the efficacy of certain entomopathogens developed as biological control agents (see references above).

Correspondence: Simon Blanford, NERC Centre for Population Biology, Imperial College, Silwood Park, Ascot, Berks, SL5 7PY, U.K. Tel: +44 (0)1344 294475; fax: +44 (0)1344 873173; e-mail: s.blanford@cabi.org

Zonocerus variegatus Linnaeus is a pyrgomorphid grasshopper of the humid tropical zone of west and central Africa. This grasshopper is a particularly well studied species (Chapman *et al*., 1986; Chiffaud & Mestre, 1990; and references therein). The increase in the number of studies on *Z. variegatus* over the last 20 years has mainly been associated with the belief that its pest status has been on the increase (FAO, 1990). Large seasonal fluctuations in population size (Chapman & Page, 1979; Chapman *et al*., 1979; Fischer & Boppré, 1997), the creation of mosaic habitats through farming practices (Page, 1978) and the spread of Siam weed (*Chromolaena odouratum*) (Fischer & Boppré, 1997), have all been implicated in the increased impact that this species has on a variety of cash and subsistence crops in west and central Africa. Reports from west African countries invariably name this species as one of the major pests against which control measures (generally chemical insecticides) have been applied (e.g. Bani, 1992; Coffi, 1992; Niassy, 1992).

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Increasingly, alternatives to chemical pesticides are being sought for locusts and grasshoppers and the LUBILOSA programme has been investigating the use of the Deuteromycete entomopathogen *Metarhizium anisopliae* var *acridum* [formerly *Metarhizium flavoviride* Gams and Rozsypal, but now reclassified (Driver *et al*., in press)] for control of a range of locust and grasshopper pests in Africa. A number of spray trials with the fungus have been made against *Z. variegatus* in southern Benin and results have been very promising (Lomer *et al*., 1993; Douro-Kpindou *et al*., 1995; Langewald *et al*., 1997). However, one of the chief criticisms directed at microbial biocontrol agents is the length of time they take to kill the target insect compared with commonly used chemical insecticides. While this criticism is not justifiable in all cases (see Bateman & Thomas, 1996; Prior & Streett, 1997), speed of kill is variable, with marked differences between laboratory and field studies possibly attributable to effects of environmental temperature and host thermal behaviour (Inglis *et al*., 1996; Blanford *et al*., 1998; Blanford & Thomas, 1999). Previous studies have suggested regulation of body temperature by *Z. variegatus* hoppers (Kaufman, 1965) and increased activity in the range 35–40 °C (Vuillaume, 1954). Given the known thermal growth profile of *M. anisopliae* var *acridum* (Thomas & Jenkins, 1997), these findings suggest that body temperature and thermoregulatory activity could provide a major constraint to pathogen development. The aim of the current investigation therefore was to determine the thermal behaviour of *Z. variegatus* and the impact that this may have on biopesticide applications of *M. anisopliae* var *acridum*.

Methods

Thermal biology studies in 1996

In March/April 1996, a population of *Z. variegatus* was studied in a 50 × 50 m fallow area in the grounds of the International Institute of Tropical Agriculture in Cotonou, Benin. The population comprised fourth- and fifth-instar hoppers and adults throughout the study period. These insects were sampled on five full days (between 27 March–8 April) between sunrise (07.00 hours) and sunset (19.00 hours) at hourly intervals. Other additional samples were also recorded over the March/April period including some night-time body temperatures.

Field site

Vegetation in the plot consisted of thin leafed, unidentified shrubs (to a height of 2 m) distributed patchily in the study area. Two areas (in one corner and along one side of the plot) had a denser concentration of these shrubs, while the majority of the study area had either individual shrubs or clumps of two or three together. Herb layer consisted of sparse grass cover to a height of 30–60 cm with numerous areas of bare soil.

Thermal biology

Recordings were made with a hand-held, single input thermometer (Omega Engineering Ltd, Manchester, U.K.) in conjunction with a fine wire copper constantan thermocouple (diameter 0.125 mm). Insects were hand-caught and the thermocouple tip inserted approximately 2 mm into the thorax. The thermometer gave fast response readings and these were taken as soon as the temperature had stabilized, generally within 7–8 s of capture. The ambient temperature at the site of capture was recorded immediately after this process. In addition to body temperature measurements, notes were made of the degree of exposure of the sampled insect to solar radiation and its height above ground prior to capture. These were simply categorized as fully exposed (full sunlight), partially exposed (dappled shade) and shaded (full shade).

Environmental conditions

Ambient temperatures were recorded on data loggers (Grant Instruments UK, Cambridge, U.K.) at a variety of heights above soil and in exposed and shaded positions within the study plot. All environmental monitoring continued throughout the study days, with recording at intervals of 10 min during the night and 1 min during the day. Ambient temperatures could then be related to the time and position that body temperatures were taken. No solar radiation monitors were available for the 1996 study and measurements of daily radiation levels were taken from the IITA weather station. Relative humidity levels were also taken from this source, which was situated 500 m away from the study plot.

Thermal biology studies in 1998 and biopesticide application

Studies in this year utilized field populations maintained in two large field arenas at the IITA station to examine the effects of thermal biology on the efficacy of a spray treatment of the *M. anisopliae* var *acridum* biopesticide.

Field site

In March/April 1998 two plots 20 × 20 m were marked out and enclosed in a plastic sheeting barrier to a height of 1 m. The inside surface of the plastic sheeting was coated with silicon paint to stop the grasshoppers climbing out. In addition, a 1.5-m wide strip running the length of the inside boundary was dug over and all vegetation removed. Each plot contained mature cassava (*Manihot esculenta*) varying in height from 1 m to 2 m. There was some difference as to the extent of the cover provided by the cassava plants, with one plot (the treated plot) having a less continuous canopy cover than the other and therefore a greater degree of regeneration by colonizing plants. Although the extent of cassava cover differed, both plots provided a mixture of food and shelter plants and a mosaic of habitats where a wide range of temperatures could be found throughout the day.

Approximately 2500 third, fourth, fifth instar and adult *Z. variegatus* were placed inside each plot and left to acclimatize for 3 days prior to the spray event. These insects were collected from a number of different field sites in southern Benin. There was a small proportion of resident hoppers and adults already in the plots when they were constructed and these were left in place. Responses to temperature by other acridid species have been shown to be similar across this age range (Lactin & Johnson, 1995).

Biopesticide application

One plot received a dose of the single spore isolate of *M. anisopliae* var *acridum* I91–609, an isolate that has been shown to be particularly virulent to *Z. variegatus* (e.g. Lomer *et al*., 1993), and one that is used within the LUBILOSA programme for controlling *Z. variegatus*. Spores were suspended in 50/50 Ondina/Shellsol, sprayed at an application dosage of 50 g/ha (2.5×10^{12} conidia/ha) and an application rate of 1 L/ha, with a hand-held Micro-ULVA sprayer (Micron Sprayer Ltd, Bromyard, U.K.). To assess the rate of infection of the fungal application in the field, insects were collected from the treated plot 24 h after spray application and monitored for mortality and signs of mycosis. A parallel sample was also collected from the control plot for comparison. These field insects were divided into two groups, one maintained in field cages in the plots (20 insects per cage with four cages in treated and control plots) and the other removed to constant environment chambers maintained at 27 °C (± 1 °C) on a LD 12:12 h cycle. Dead insects were removed from both treatments each day and placed in incubation boxes (100% r.h) to encourage sporulation.

Thermal biology

Body temperature were recorded as above, except that enclosure populations were monitored every 2 h (06.00–20.00 hours) from one day before application and for the following 8 days. Transects across the enclosures were walked until sufficient data points had been collected (10–15 per sample). *Environmental conditions*. Environmental temperature was measured as above. Solar intensity was recorded using a silicon cell Pyranometer (Skye Instruments Ltd, Llandrindod Wells, UK) with one of the data loggers. Wind speeds were measured outside the plots at 1 m above ground level. All environmental temperature and solar radiation measurements were taken within the study enclosures

Light bulb cage studies

In addition, to investigate in more detail whether infection caused a change in thermal behaviour, cage studies were also conducted to assess any change in preferred temperature of insects infected with fungal conidia from the spray application compared with control insects. Thirty third- and fourth-instar hoppers (15 from treated plot and 15 from the control plot) were collected one day after the spray event and were placed in separate cages in the laboratory. Cages (50\cm × 50 cm × 50 cm) had a 60 watt light bulb fixed 3/4 of the way up the back wall. Cage sides consisted of 2 mm wire mesh and a wire mesh tube was also placed in the cage, positioned in front of the light bulb. The tube (diameter approximately 14 cm) reached from the top to the bottom of the cage and provided a variety of perching sites in front of the light bulb. Thus, grasshoppers could move and perch within a thermal gradient created by the light bulb (46–48 °C directly above the bulb to 30–32 °C furthest away from the bulb). Light bulbs were switched on for 12 h each day (08.00–20.00 hours). Measurements of the temperature at the insects' perching position were made with the fine wire thermocouples and hand-held thermometer described above. The thermocouple tip was lowered through the mesh so that it was as close as possible to the midpoint of the insects thorax. Thus, the perching site temperature was measured rather than the actual body temperature. Monitoring commenced 1 day after application and for the following 6 days. Each group was monitored four times each day at two-hourly intervals (10.00–16.00 hours).

To compliment the cage gradient study on control and field sprayed hoppers and ensure that all treated insects were infected, adult *Z. variegatus* were hand-inoculated with a known dose of *M. anisopliae* var *acridum* and confined in the same cages (12 control and 12 treated). Inoculation took place using oil suspensions of *M. anisopliae* var *acridum*, isolate (I91–609), formulated and applied following a similar technique to that described by Prior *et al*. (1995). Spores were collected from Sabouraud dextrose agar (SDA) slopes and formulated in groundnut oil. These fresh suspensions were placed in a bath sonicator for 1 min to break up the conidial chains and conidial counts were made with a haemocytometer. Each grasshopper in the treatment groups received 1 μL of conidial suspension applied with a micropipette beneath the dorsal pronotal shield. Spore concentrations in the formulations were adjusted to give 1×10^{3} spores per insect. Control adults remained untreated, as previous studies have shown the blank oil formulation to have negligible effects on mortality of grasshopper populations in the laboratory and field (Lomer *et al*., 1997). Both treated and control grasshoppers were handled in a similar way when assigned cages except for the fact that treated individuals received the droplet of conidial suspension. Monitoring of these adult grasshoppers commenced one day after inoculation and continued for the following 6 days. Temperature measurements of selected perching position were carried out in the same manner as described above at the same time interval.

Results

Thermal biology studies – 1996

Weather conditions. Mean ambient temperatures during the day at a variety of positions in the environment are shown in Table 1, together with mean relative humidity monitored in the field. Solar radiation is taken from the IITA weather station for the days on which monitoring was carried out. Each day was characteristically hot and humid, with soil surface temperatures regularly reaching 50 °C or more. Temperatures from 1 m and 2 m above the ground varied little between the two positions and

Table 1 Mean (± SE) exposed ambient temperature (07.00–19.00 hours) at three different positions during 1996. Relative humidity is the mean (± SE) of three full days monitoring and solar radiation levels (g-calories m^{-2}) were provided by the weather station in the IITA grounds close to the study sight.

	Mean (± SE)	Max	Min
Soil surface (°C)	41.2 (± 1.21)	57.2	27.6
1 m (°C)	32.3 (± 0.39)	36.4	26.4
2 m (°C)	32.1 (± 0.40)	36.4	26.4
Relative humidity (%)	77.9 (± 1.64)	98	47
Solar radiation (g-cal/cm^{-2}/day)	289.0 (± 6.34)	329.3	240.2

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were generally 33–36 °C. Relative humidity fell during the day to 50–65%, whereas at night, humidity was consistently over 90%.

General observations. Grasshoppers could be found in exposed roosting positions at the beginning of the day. Generally these were at the top of the roosting shrubs on the exposed surface of leaves. Following sunrise (07.00 hours) grasshoppers gradually moved to feeding sites or commenced feeding on plants that they had roosted on. No descent to soil surfaces, ground grouping or basking behaviours were ever observed at this time, even though exposed soil surfaces were available throughout the study area. Throughout the day, insects could be found at a variety of heights above ground but very few were ever sampled on the ground ($< 1\%$, $n = 530$) and, similarly, very few remained in the exposed roosting sites. Grasshoppers were very sedentary and remained in the study area throughout the sampling period. During the hottest part of the day (10.00–16.00 hours) most grasshoppers exhibited heat avoidance behaviours. These included shade seeking (underside of leaves or descent into the main body of the vegetation) and minimization of body surfaces in touch with vegetation. This stilting behaviour (usually observed in grasshoppers and locusts on the ground) involved pushing the body away from the plant stem and holding on with only the first two pairs of legs. Towards evening (18.00 hours) grasshoppers re-ascended to the top of shrubs for roosting. Once again, no ground grouping or basking behaviours were observed.

Body temperature. Pooled body temperature data from the 5 days are shown as an hourly body temperature profile in Fig. 1. Mean body temperature (± SE) between 07.00 and 19.00 hours was found to be 34.5 (± 0.23) °C (max. 45.9, min. 23.7, $n = 530$). Between 10.00 and 16 .00 hours, the period where heat avoidance behaviours could be observed, mean body temperature was 37.7 (± 0.22) °C, $n = 218$. Night-time body temperatures were found to be close to, though slightly above, ambient temperatures in the range 27.2–30.1 °C between 22.00 and 05.30 hours [mean (± SE) ambient temperature = 25.8 (± 0.76) °C, body temperature = 28.7 (± 0.14) °C, $n = 41$].

Thermal biology studies – 1998

Weather conditions. Data for night time and day time temperatures, similar to those above, are given in Tables 2a and b. Mean solar radiation during 1998 was 443.24 (± 22.669) W m^{-2} (max. 1067.83, min 1.33) between 07.00 and 19.00 hours. Wind speed was generally in the range 1–6 m/s with gusts of 11–12 m/s. Generally, early morning wind speeds were very low (< 1 m/s 05.00–08.00 hours). Relative humidity averaged 75.2 (± 1.32)% with the highest levels recorded at night and during the early morning before sunrise (95% or greater) and the lowest levels found during the early afternoon (range 52–71%).

General observations. The observed pattern of behaviour between the 1998 population and the 1996 population (above) was very similar. Grasshoppers could be found in exposed roosting sites in the early morning, from which they gradually dispersed to feeding sites either on the same plant or other plants regenerating in different areas. Many individuals were associated with *Chromolaena odouratum*, for which this species has a special attraction (Fischer & Boppré, 1997) and which were present in both treated and control enclosures. Shade-seeking behaviour was observed in both the 1998 enclosures but the elaborate cooling behaviour described for the 1996 population was never recorded (for description of grasshopper cooling behaviours see Chappell & Whitman, 1990). At no time were any overt basking or sun-seeking behaviours observed, nor were any postural adjustments associated with heat gain observed. No ground-basking groups formed on any day (morning or evening) even though the cleared strips at the edge of the enclosures provided an ideal resource for such behaviour.

Body temperature. When all days were pooled a significant difference between body temperature of control and treated grasshoppers was apparent ($F_{1,1162} = 7.34$, $P = 0.0068$). Day-by-day comparison indicated a significant difference between treatments on different days but no difference when control and treated groups were compared on the same day ($F_{17,1161} = 4.18$, $P < 0.001$; Tukey HSD $P > 0.05$ for control and treated on the same day between 06.00 and 20.00 hours). Daily mean body temperature varied between 31.8 (± 0.24) °C and 34.0 (± 0.31) °C for control samples (range 25.1–38.3 °C) and 32.1 (± 0.32) °C and 34.8 (± 0.35) °C for treated samples (range 26.3–40.3 °C). Mean difference between average control and treated body temperatures on the same day was 0.52 (± 0.098) °C. During the hottest period of the day (10.00–16.00 hours as above), when any difference in thermal behaviour is likely to be accentuated, differences in mean body temperature were apparent on days 3 and 6 [($F_{17,620} = 15.92$, $P < 0.001$; Tukey HSD $P < 0.05$; mean (± SE) body temperature for control 32.9 (± 0.50) °C and treated 33.8 (± 0.57) °C on day 3 and control 32.4 (± 0.34) °C and treated 33.1 (± 0.39) °C on day 6, respectively)] but not on any other day. Figure 2 indicates that treated insects had higher mean body temperatures than control insects across the study period.

Spray mortality. Cumulative proportional survival for grasshoppers maintained in the laboratory and the field are shown in Fig. 3. Treated, field-cage maintained grasshoppers began to die on day 6, and by day 10 (end of monitored period) mortality had reached 45%. Grasshoppers sprayed in the field and removed to

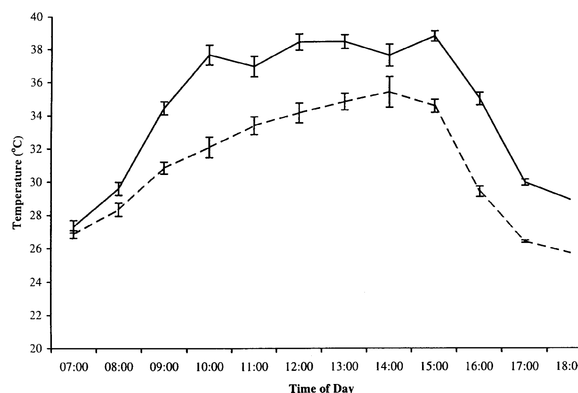

Figure 1 Mean (± SE) hourly body temperature of *Z. variegatus* (solid line) and mean (± SE) hourly ambient temperature at 1 m in exposed position (dashed line) for the 1996 field population. Each hourly mean body temperature represents a minimum of 32 individual readings.

Table 2 Mean (± SE) ambient temperatures during the 1998 spray trial. (a) Temperatures were recorded at a variety of heights for both day and night in exposed positions. Means are taken from continuous data logged during the monitoring period**.** (b) Temperatures at a variety of heights for both day and night in shaded positions (under cassava canopy in the control plot). Means in both tables are taken from continuous data logging during the monitoring period. 'Day' = 07.00–19.00 hours, 'Night' = 19.00–07.00 hours.

	Temperatures (°C)			
	Soil surface	0.5 m	1 m	2 m
(a) Shade				
24 h (± SE)	31.6 (± 0.034)	30.5 (± 0.029)	30.5 (± 0.029)	30.2 (± 0.029)
Max, Min.	41.2, 24.8	38.8, 21.0	38.4, 22.0	38, 21.6
Day (± SE)	34.7 (± 0.041)	33.2 (± 0.031)	33.2 (± 0.032)	32.9 (± 0.032)
Max, Min.	41.2, 28.8	38.8, 27.8	38.4, 27.8	38, 27.6
Night (± SE)	29.4 (± 0.019)	28.5 (± 0.025)	28.4 (± 0.027)	28.2 (± 0.026)
Max, Min.	31.4, 24.8	30.4, 21.0	30.4, 22.0	30.0, 21.6
(b) Exposed				
24 h (± SE)	36.2 (± 0.67)	30.1 (± 0.21)	29.7 (± 0.18)	29.7 (± 0.20)
Max, Min.	65.8, 23.8	37.1, 20.9	35.5, 21.6	35.9, 21.9
Day (± SE)	43.9 (± 1.02)	32.5 (± 0.27)	31.7 (± 0.26)	31.8 (± 0.27)
Max, Min.	67.6, 25.13	39, 24.0	37.2, 23.6	37.4, 23.2
Night (± SE)	29.4 (± 0.24)	28.1 (± 0.18)	28.0 (± 0.17)	27.8 (± 0.20)
Max, Min.	39.0, 23.8	32.3, 20.9	31.6, 21.6	31.7, 21.9

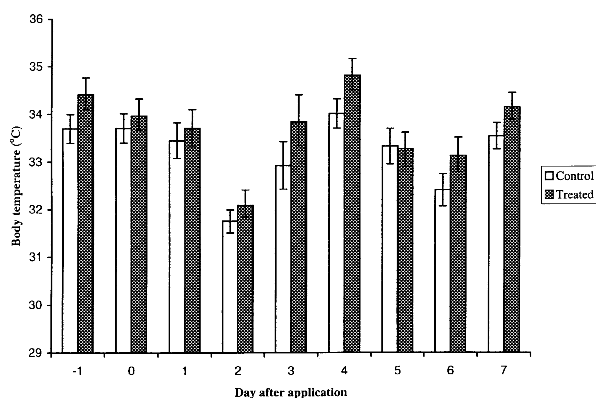

Figure 2 Daily mean (± SE) body temperature of control and treated *Z. variegatus* from the 1998 field trial. Body temperatures were recorded at two-hourly intervals. Spray application occurred on day 0. Each mean hourly body temperature represents a minimum of 51 readings.

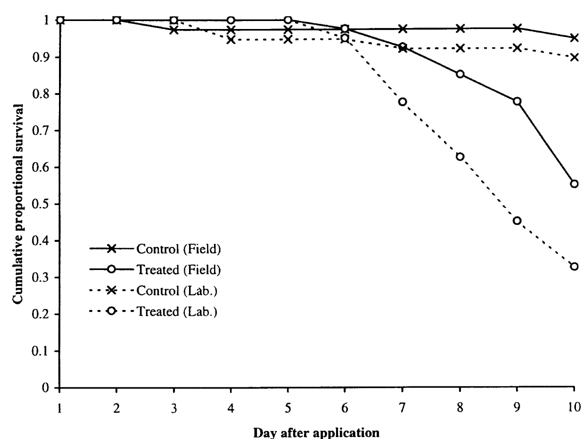

Figure 3 Cumulative proportional survival of *Z. variegatus* following application of *M. anisopliae* var *acridum*. Control and treated insects were either maintained in field cages (Field) or removed to a constant environment chamber in the laboratory (Lab.).

the constant environment chambers began to die on day 4 and mortality had reached 67% by day 10. Insects dying in these treatments showed characteristic red coloration, indicative of *M. anisopliae* var *acridum* infections and external sporulation when incubated at high humidities. Control mortality was low (5 and 10% for field-maintained and laboratory-maintained samples, respectively), with none showing any signs of infection by *M. anisopliae* var *acridum*. Kaplain–Meier Survival analysis gave average survival times (± SE) of 9.82 (± 0.26); 9.61 (± 0.26); 9.53 (± 0.16) and 8.80 (± 0.21) days for control field sample, control laboratory-maintained sample, treated field-maintained sample and the treated laboratory-maintained sample, respectively. There were significant differences in survival time between the laboratory-maintained control and treated groups ($P < 0.0001$) and the field caged-maintained control and treated samples ($P < 0.001$). There was also a difference between laboratory- and field-maintained treated samples ($P = 0.012$) but no differences between laboratory- and field-maintained controls ($P = 0.40$).

Light bulb cage study. Analysis of variance with repeated measures indicated no significant difference between selected perching positions by control and treated adult *Z. variegatus* ($F_{7,1} = 1.03$, $P = 0.343$). Mean (± SE) temperature of selected perching positions was 33.8 (± 0.067) °C, maximum 37.6 °C, minimum 31.0 °C, and 34.0 (± 0.070) °C, maximum 39.9 °C, minimum 30.4 °C for control and treated adults, respectively, and this was supported through direct observation. None of the grasshoppers displayed any thermally mediated movement towards the light bulb, no postural adjustments were observed and, in general, grasshoppers appeared to be distributed randomly around the cage. Similarly, for field-sprayed hoppers confined in the light bulb cages there was no significant difference in perching position ($F_{7,1} = 5.14$, $P = 0.058$). Mean (± SE) temperature of selected perching position was 34.1 (± 0.090) °C, maximum 37.3 °C, minimum 31.0 °C and 34.0 (± 0.091) °C, maximum 36.7 °C, minimum 30.9 °C, for control and treated hoppers, respectively. Direct observation of the position of hoppers in the cages also indicated that there was no aggregation around the light bulb. Cage temperatures were

fairly uniform except a short distance away from the bulb and were slightly lower than measured field temperatures, being generally in the range 31–35 °C. Nevertheless, neither hand-inoculated adults nor sprayed hoppers showed preference for hotter temperatures around the bulb.

Three adult treated grasshoppers (25%) and no control adult grasshoppers died during this cage study. Five hoppers from the treated cage (33%) and two control hoppers died during the assessment period. All adults and four of five treated hoppers expressed *M. anisopliae var acridum* infection when incubated at high humidity, whereas no control cadavers indicated that death was caused by the fungus.

Comparison between 1996 and 1998

Environmental conditions. Environmental data from the IITA weather station indicated that there was no difference in mean temperature ($t = 0.21$, d.f. = 24, $P = 0.83$) between study periods in 1996 and 1998. However, there were differences in both solar radiation ($t = 3.16$, d.f. = 24, $P = 0.004$: mean solar radiation 1996 – 296.7 (± 6.79) g/cal/day; 1998 – 375.1 (± 23.88) g/cal/day) and relative humidity ($t = 2.43$, d.f. = 24, $P = 0.02$: mean r.h. for 1996 – 76.9 (± 0.53)%; 1998 – 74.9 (± 0.61)%. Ambient temperature measured at the field sites in 1996 and 1998 also showed no difference between the two [$t = 0.66$, d.f. = 37, $P = 0.51$; mean (± SE)]; ambient temperatures during the study periods were 30.1 (± 0.99) and 30.9 (± 0.57) °C for 1996 and 1998, respectively. A significant difference was found when ambient temperature at the site of capture was measured between study groups in 1996 and 1998 ($F_{2,1687} = 39.9$, $P < 0.001$) but not between control and treated groups in 1998 (Tukey HSD $P > 0.05$). When the degree of exposure of individual insects was compared, the 1996 sample and the 1998 treated group were generally found in exposed positions more often (58% and 57%, respectively) than the control group, 34% ($\chi^2 = 11.59$, d.f. = 1, $P < 0.001$ vs. 1996 sample, and $\chi^2 = 10.67$, d.f. = 1, $P < 0.001$ vs. treated). Body temperatures of 35 °C or greater were reached more often in the 1996 group (52.5% $n = 530$) followed by the treated group (44.9% $n = 559$) and the control group (35.6% $n = 553$) ($\chi^2 = 31.13$, d.f. = 1, $P < 0.001$ for 1996 group and control 1998 group). Body temperatures over 40 °C were never reached by the 1998 control group, rarely in the 1998 treated group (0.35%), but more often in the 1996 group (11.5%).

Body temperature. Given that ambient temperature did not differ from either weather station data or site temperature and that capture site may have been influenced by microclimate features and/or insect behaviour the field population in 1996 and the control and treated group in 1998 were compared. This revealed that the 1996 population maintained mean body temperatures higher than 1998 samples between 10.00 and 16.00 hours, when the highest body temperatures could be reached (Fig. 4a). Pooled mean body temperatures for this period were, 1998 control 35.4 (± 0.086) °C, $n = 307$, 1998 treated 36.1 (± 0.94) °C, $n = 314$, 1996 sample 37.7 (± 0.22) °C, $n = 218$. One-way analysis of variance between the three groups revealed a highly significant difference ($F_{2,836} = 58.89$, $P < 0.0001$, Tukey HSD $P < 0.05$ for all groups). Linear regression of body temperature on ambient for all three groups is shown in Fig. 4(b). Slopes of each regression were also significantly different between each group ($t = 8.14$, d.f. = 529, $P < 0.001$ for 1996 vs. control 1998; $t = 5.23$, d.f. = 529, $P < 0.001$ for 1996 and treated 1998; $t = 2.37$, d.f. = 578, $P < 0.05$ for control 1998 vs. treated 1998).

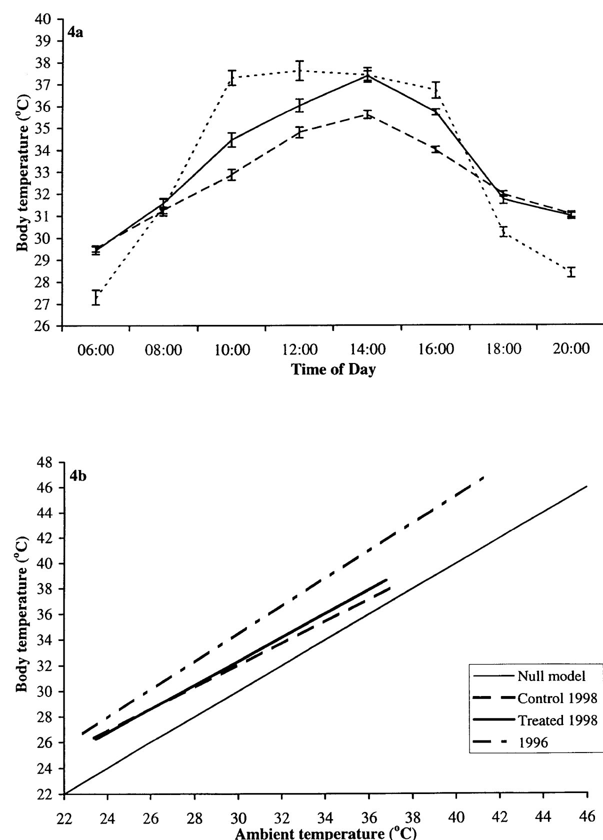

Figure 4 Body temperature profiles and regression curves for *Z. variegatus*. (a) Comparison of pooled mean (± SE) hourly body temperature for the 1996 field population (dotted line) and the 1998 control (dashed line) and treated (solid line) samples. Two-hourly means for the 1996 field population represent a minimum of 30 readings and hourly means for the 1998 samples represent a minimum of 90 readings. (b) Distribution of body temperature (Tb) against ambient temperature (Ta) for *Z. variegatus.* The null model indicates equilibrium where Tb = Ta. Curves show best fit linear regression describing the pattern of body temperature for each study group. Regression equations are $Tb = 1.0944Ta + 4.363$, $R^2 = 0.84$ for 1996; $Tb = 0.8621Ta + 6.1718$, $R^2 = 0.78$ for control 1998; $Tb = 0.9323Ta + 4.363$, $R^2 = 0.74$ for treated 1998. Data points have been omitted for greater clarity.

Discussion

Vuillaume (1954) described the preferred temperature of *Z. variegatus* as 36–40 °C. This range was attained from observations of activity patterns across a range of temperatures and, as such, was not an examination of behavioural thermoregulation. Kaufmann (1965) described *Z. variegatus* basking following descent from roosting sites. However, no further comments were made on the specificity of this basking behaviour (orientation to the sun, etc.) and no further observations were made of similar behaviour in the evening. Basking on soil surfaces is characteristic of many actively thermoregulating acridids

(Uvarov, 1977) but this behaviour invariably occurs in the morning following descent from roosting sites and in the evening prior to ascent to the latter sites. Thus, neither report can be said to have ascertained any clear thermoregulatory behaviour by *Z. variegatus.* In this study *Z. variegatus* did show shade-seeking and heat avoidance postures (particularly in the 1996 group), but this was far from the full range of active thermoregulatory behaviours exemplified by other acridids (e.g. Chapman, 1965; Lactin & Johnson, 1996, 1998; Blanford *et al*., 1998). In addition, unlike a range of other species [*O. senegalensis* (Blanford *et al*., 1998); *Locustana pardalina* (authors' unpublished data); *Schistocerca gregaria* and *Dociostaurus maroccanus* (Blanford & Thomas, 1999)], individuals showed no clear 'behavioural fever' in response to infection in the cage gradient. Although not conclusive without further ecophysiological studies, *Z. variegatus* does not appear to actively regulate its body temperature in order to attain and maintain a preferred body temperature.

All three study groups showed differences in both the highest body temperatures recorded and the length of time that these were maintained during the day. The 1996 study area was clearly more sparsely vegetated than either of the 1998 study plots. The increased likelihood of exposure to solar radiation and elevated ambient temperature in this plot may account for the higher body temperatures and increased heat avoidance behaviours found for the 1996 group. In 1998, plots were chosen that had a similar feeding (i.e. adequate food plants) and thermal resources (shade and exposed areas). Nevertheless, there were still vegetation differences that, in retrospect, may have altered *Z. variegatus* degree of exposure to environmental conditions. An insect that regulates body temperature could have moved to, and utilized, advantageous microhabitats (e.g. cleared soil surfaces bordering the plots) to achieve a preferred body temperature. However, in this case *Z. variegatus*, interacting passively with ambient temperature and solar radiation, would reflect, via its body temperature, the likelihood of being in exposed or shaded microhabitats. Thus, it would appear that even slight differences in habitat structure (the treated plot having a less continuous canopy than the control plot) increase the likelihood of grasshoppers being exposed to solar radiation. This may explain not only the difference between the 1996 and 1998 groups but also the generally higher body temperature experience by the treated group in comparison to the control group in 1998. Such a finding has important considerations for pathogenic disease development.

In other studies it has been noted that elevated body temperature via active behavioural thermoregulation confers considerable advantages to acridids infected with entomopathogenic fungi (Carruthers *et al*., 1992; Inglis *et al*., 1996). *Zonocerus variegatus*, exhibiting no active body temperature regulation, could be considered to be more susceptible to infections caused by these pathogens. However, body temperatures achieved by *Z. variegatus* in this study are at a level that would inhibit disease development by *Beauveria bassiana* (Balsamo) Vuillemin and *Entomophaga grylli* (Fresenius). Both these fungal pathogens have upper limits for growth of 35 °C and have received considerable attention as candidate microbial agents for grasshopper control (Carruthers *et al*., 1992; Inglis *et al*., 1996). Indeed, *E. grylli*, a naturally occurring pathogen of *Z. variegatus* and one that causes significant mortality in wet season populations (Chapman & Page, 1979; Chapman *et al*., 1979; Chapman *et al*., 1986), can be eliminated from host haemocoel if body temperature is maintained at 35 °C or higher for a number of hours (Carruthers *et al*., 1992). Therefore, protection from entomopathogenic fungi via passively acquired body temperature may be a significant factor in determining the success of pathogenic infections both through natural routes of contact or those that are picked up from a biological control application.

Spray applications of *M. anisopliae* var *acridum* that directly target populations of *Z. variegatus* result in high mortality (Lomer *et al*., 1993; Douro-Kpindou *et al*., 1995; Langewald *et al*., 1997). In this study, mortality was also rapid and would likely have been high if the assessment had continued for a longer period. The chief reason may be that *M. anisopliae* var *acridum* is more tolerant of elevated temperatures than a number of other fungal pathogens; it does not completely stop growth until temperatures reach 40 °C or higher (Thomas & Jenkins, 1997). That said, there was still a significant difference between grasshoppers maintained under constant temperatures in the laboratory and those held in field cages in the study plots. Thus, speed of kill is likely to alter depending on environmental conditions (e.g. factors that influence heat acquisition such as cloudy days). Moreover, it is also apparent that mortality rate will vary between habitats even under the same environmental conditions because subtle differences in habitat structure, such as those between field plots in the 1998 study, may affect body temperature profiles significantly. This could have a significant impact on the length of time the pathogen takes to effect control in the field.

Such differences in body temperature, apparent in plots only 500 m apart, highlight the complexity involved in predicting the outcome of control campaigns using fungal pathogens.

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