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Fever impacts on host life history traits, but is this a cost?

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ABSTRACT
Fever is one mechanism by which animals may defend themselves against pathogens but, as with other defenses, may have consequences for the host. In ectotherms such as the desert locust (*Schistocerca gregaria*), fever temperatures are attained through modified behavioural thermoregulation. We have previously demonstrated that the fitness benefits of behavioural fever in *S. gregaria* can be substantial: a fever temperature just 2-4°C higher than the normal can make the difference between zero and near-normal reproduction when infected with a fungal pathogen. Here we examined whether there are costs associated with these elevated temperatures by holding adult, gregarious *S. gregaria* at elevated temperatures for one or five hours per day and for ten or twenty days. We found that, while fever temperatures did affect locust life history traits, this was expressed through the ability of locusts to sustain flight and to compete for mates, rather than the primary fitness correlates of survival and fecundity. In addition, there was no relation between time spent at fever temperatures and magnitude of the response. While these effects could result from production of molecular chaperones, for example, indicating a direct cost of fever, they are also consistent with an effect of fever on the locust hormonal system and a shift towards the *solitaria* phase state. Although this can still be interpreted as a cost of fever, in the field context, a shift towards solitary behaviour could also be viewed as adaptive life history response to limit the impact of disease. These conflicting interpretations highlight the need for careful consideration in identifying response traits and the importance of considering complex defence mechanisms and trade-offs in an appropriate ecological context.

Key-words: Behavioural fever, Costs of resistance, Induced defense, Host-pathogen interactions, Ectotherms, Locusts, Entomopathogenic fungi, Phase change.

INTRODUCTION

The insect immune system has recently received much attention from evolutionary ecologists, both as a counterpoint to the vertebrate immune system, and as a tractable means to test hypotheses relating to the costs of mounting a defense to pathogens (1-3). Current theory is that costs of defense can be reduced by (and so select for) inducibility and specificity (4, 5) and through adaptive changes in life history of the host (3). One component of the immune system which has been little considered in this light is fever, although it is a common defense response, with apparently well-conserved physiological mechanisms, in a diversity of invertebrate and vertebrate taxa (6, 7). Within the evolutionary ecology framework of host-pathogen interactions, fever is clearly an induced defense and can also have a degree of specificity (8). What, then, are the costs of this defense response?

An increase in an animal's body temperature above the normal set point would be likely to carry costs (9). Indeed, fever can be lethal (notably in mammals), which has led to a substantial debate as to whether, in fact, fever should be regarded as an adaptive defense mechanism at all (9-11). In many, though not all, ectotherms such as reptiles or insects, thermoregulation is achieved by behavioural adjustment in the local environment to enable heat loss or gain. When infected, these ectotherms may alter thermoregulatory behaviour to shift their normally preferred body temperature to a higher set point in what is termed "behavioural fever" (12-14). Using desert locusts (*Schistocerca gregaria* (Forskål)) and the mitosporic fungal pathogen *Metarhizium anisopliae* var. *acridum* (*Metarhizium flavoviride* Gams and Rozsypal (15)), we have demonstrated that behavioural fever can be an essential defense response to combat disease: the ability to attain a fever temperature just 2-4°C higher than the normal set point body temperature can make the difference between zero and near-normal reproduction (16). In addition to this, however, we have recently demonstrated that, following exposure to fever temperatures – both through active thermoregulation in the presence of a pathogen and via imposed thermal conditions in the absence of a pathogen – *gregaria* phase *S. gregaria* adults produce more of the phenotypic *solitaria* phase state offspring (versus *gregaria*) than do parents that are not subjected to fever regimes (17). This shift in offspring phase state was unexpected given that the range of mechanical, visual and olfactory cues, which determine phase state (18) were controlled between treatments and were consistent with a high density of conspecifics.

Thus, there is evidence for fever in desert locusts producing a (trans-generational) change in life history, though whether this effect can be defined as a cost and what the mechanisms are remains unclear. Here, we further explore the effects of fever by manipulating locust body temperatures and investigating the effects of a range of simulated fever conditions on both gross fitness measures (survival and fecundity) and on more subtle measures (mate guarding and flight capacity). Our hypothesis was that if fever temperatures are costly, we should see effects on some aspect of fitness and that these costs should vary depending on the pattern and intensity of exposure to fever temperatures.

MATERIALS AND METHODS

Overview

We held mixed-sex groups of newly moulted adult locusts for 20 days (the sexual maturation phase) at temperatures representative of normal behavioural thermoregulation: 20°C at night and 38°C during the day. For some of these groups, we elevated the temperatures slightly to simulate behavioural fever: 20°C at night, 38°C for most of the day but with an additional fever temperature of 42-44°C for part of this period (16, 19-21). Based on previous observations (authors' unpublished data) we subjected the locusts to fever temperature for a conservative 1hr. /day, or alternatively a more extreme 5 hrs. /day. This was done for the first ten days of the maturation phase, the second ten days, or all twenty, to represent shorter- or longer-term fever responses at different host ages. We then separated the sexes and assessed a range of fitness correlates. By these means, we investigated the cost of fever temperatures in *S. gregaria* in isolation from potential consequences of infection and associated immune responses, and without the invasive use of pyrogens which would compromise longer-term assessment of fitness.

Experimental treatments

Schistocerca gregaria were obtained as 5th instars (Blades Biological, Edenbridge, Kent, UK). They were initially maintained in standard aluminium locust cages, with 40W light bulbs on the back wall and a plastic mesh climbing frame to allow thermoregulation. Except where stated, locusts were fed *ad libitum* on ca. 14 day old wheat seedlings and bran. Locusts not used as the principal test animals were maintained in these conditions and are henceforth referred to as "stock" males or females. After moult, adults of 0-3 days old were taken from the stock cages and placed in opaque plastic cages (22cm x 15.5cm x 11.5cm). These were ventilated in the top and sides with muslin, with wire mesh inside to allow climbing. A replicate consisted of one such cage with 7 females and 5 males. Five blocks comprised seven such replicate cages each (see treatments below). To account for the natural distribution in moulting, blocks were staggered so that all locusts were placed in the treatments at a similar physiological age.

The cages were maintained in a climate room on a 9L:15D cycle. For the first and last hours of the light phase, temperature and fluorescent lighting ramped linearly up or down (respectively) to simulate dawn and dusk. Temperatures were set at 20±1ºC in the dark phase (16). The light phase temperature was set at 40ºC (i.e. seven hours accounting for ramping) which created a thermal gradient in the climate room from c. 37°C on the lower shelving, to c.44°C on the upper shelves. This natural gradient was used to simulate either normal thermoregulatory temperatures (ca. 38-39°C) or behavioural fever temperatures (42-44°C). More specifically, the room was 'mapped' using a digital temperature logger and 35 positions were marked out in which daytime temperature was 37.4ºC-38.9ºC. These positions were used to maintain locusts at normal thermoregulatory temperature. A further 20 positions were similarly located in which daytime temperature was 42ºC-43.8ºC. To simulate a range of fever responses, cages were placed in these "fever" positions for either 1 or 5 hours per day (in the middle of the light phase) during days 1-10, 11-20 or 1-20 of the adult locust maturation phase. Cage positions were assigned randomly. Control cages remained at normal thermoregulatory temperature but were moved randomly among the 35 suitable positions each day.

Fitness correlates

The primary correlates of locust fitness we assessed were survival time and fecundity. In addition, the mating competitiveness of males/acceptance by females (i.e. the ability of males from the different treatments to mount control females and remain there in the face of competition in combination with their acceptance by those females) and the ability of males to sustain flight were assessed as more subtle fitness correlates.

Following the first phase of the experiment, females from the different treatments were returned to standard locust cages, with 40W light bulbs to allow thermoregulation. Ten stock males (see above) were added to each cage: five mature adults and five nearing maturity (stage 3 on a visual colour score (22)) providing females with a range of mating partners. Food was made available for only one in three days to induce a degree of stress which might exacerbate differences in condition between treatments.

Female deaths were recorded each day and these data were subjected to Kaplan-Meier survival analysis (SPSS for Windows v. 6.1).

Two plastic cups (each 12 × 6cm) of moist silver sand were supplied in each cage for oviposition. These were changed every 3-4 days. Once removed, the cups were moistened with distilled water, covered with plastic bags and incubated at 30±1ºC. Bags into which hatchlings had emerged were removed daily and were frozen. Hatchlings were subsequently counted and analysed with a Kruskal-Wallis test as irresolvable heteroscedasticity did not permit parametric statistics.

Males were retained in the plastic boxes once the females had been removed, and were maintained at 30±1ºC. 5 female and 5 male stock locusts were added to each cage of 5 treated locusts. To differentiate between treated and stock males, locusts were marked with Tippex™, with either a stripe or two small dots. Preliminary tests showed the markings to have no effect on the locust's mate choice or survival. Again, food was made available every third day. Thrice daily, observations were made of which males were successfully guarding a female (i.e. sitting on her back), giving paired observations of mate guarding by treated versus stock males. These observations were made for 14 days and were then pooled for sign tests (N = 201).

Between 16 and 24 days post-treatment the ability of the treated male locusts to sustain flight was assessed in a wind tunnel (23). The locusts were flown in batches of 7, one from each treatment, repeated four times for each replicate (as far as mortality within replicates allowed), starting with the first block so that all males were flown at a similar physiological age. The locusts were attached to metal rods using a loop of nylon tied just behind the front legs and over the pronotum, to avoid interference with the wing movement. The locusts were flown in a line facing the wind source - the position for each treatment being assigned randomly. The wind speed was set to 3.9 ±0.1 m/s (under these conditions the locusts are able to sustain periods of continuous flight (23)) and run for three hours continuously. Every 30 minutes, locusts were observed for five one minute intervals to record whether they were flying or not, giving a total of 30 counts for each locust. These data were analysed using linear mixed effects models in R version 1.5 - (ref. 24, pf. 659)) to account for repeated measures, with time as the primary covariate and block as the grouping factor.

Following the flight experiment, males were kept separately in plastic pots and at 27 days post-treatment, provision of food was stopped. Mortality was recorded daily and analysed as above.

RESULTS

There were no significant differences between the treatments in the mean survival times of either male or female locusts (Kaplan-Meier survival analyses with log-rank statistics accounting for block 0.00 to 2.44, $P > 0.05$, Fig. 1a,b). Similarly, there were no effects of treatment on the fecundity of the female locusts (Kruskal-Wallis $P = 0.807$, d.f. = 6, Fig 1c). The other, more subtle fitness correlates did, however, show significant treatment effects. Repeated observations of which males were mounted on females revealed that, except for one treatment, there was an overall tendency for the males from the fever regimes to be less competitive than their untreated counterparts (sign tests, $P < 0.05$, Fig. 1d). Moreover, observations of the ability of males to fly in a wind tunnel post treatment showed that control males were 15-25% better than those subjected to fever temperatures in sustaining flight over a 3 hour period (linear mixed effects model, $P < 0.001$, $F_{1,8} = 15.02$, Fig. 1e). However, as with mating competitiveness, there were no differences between fever treatments, indicating no obvious effect of intensity of fever on fitness (linear mixed effects models, deletion of second ten days as a factor: $P = 0.719$, $F_{1,14} = 0.129$; deletion of first ten days as factor: $P = 0.167$, $F_{1,11} = 1.910$; deletion of intensity: $P = 0.635$, $F_{1,8} = 0.225$).

DISCUSSION

We hypothesised that there would be life history side effects (costs) associated with exposure to fever temperatures and that these would depend on the degree of exposure. In line with the first part of this hypothesis, our results revealed impacts of fever temperatures on flight and mating behaviour, though not on primary fitness correlates such as survival and fecundity (Fig. 1). While these results contrast with several other studies which report physiological linkages between immune stimulation and primary traits such as fecundity (e.g. 25, 26), they do, nonetheless, demonstrate a measurable effect of fever on host life history and add to a growing body of literature indicating that consequences of defense may be expressed through a variety of fitness measures (4, 26-33). Contrary to the second part of our hypothesis, however, the effects were detectable with a total of only 10 hours of elevated temperature over 10 days and were insensitive to changes in the pattern or intensity of fever.

The increase in metabolic rate and consequent energetic costs associated with fever temperatures can be substantial (e.g. 34, 35). In addition, an almost universal response to heat stress is the production of protective molecular chaperones such as heat shock proteins (Hsps) (36, 37) or trehalose (38). Producing these chaperones, and subsequently breaking them down, would have an associated cost and might provide the basis for the side effects of fever we observe in the locust fitness traits. Moreover, acclimation to higher temperatures can lead to higher standing titres of Hsps (39, 40), which might explain the one-off threshold cost we observe, rather than any increase in costs or side-effects as intensity of fever increases (although this is not the only explanation as studies using other immune stimulation techniques have also reported insensitivity in the response of life history traits to 'dose' (e.g. 26). Furthermore, fever in locusts has been shown to increase mobilisation of haemocytes and anti-pathogen metabolites (41). This again would be expected to carry energetic costs or trade-offs, although it is not clear whether in the absence pathogens or other pyrogenic material, elevated temperature alone is sufficient to initiate the response (7). Thus, we can identify possible physiological or biochemical responses to elevated temperature that might be expressed as costs. Why these should trade-off against subtle traits such as mating behaviour, rather than primary fitness correlates such as fecundity is, however, unclear. In this respect, an alternative (although not necessarily mutually exclusive) hypothesis based on effects of temperature on locust phase state is, we believe, more compelling.

Flight performance is one of a range of behaviours that differ between solitary and gregarious locusts. Several studies implicate adipokinetic hormone (AKH) in flight performance, since it modulates the transport of lipids, the major fuel for long distance flight (42), to the flight muscles. For example, isolation of gregarious male locusts for 14 days from a previously gregarious stock alters their ability to mobilise lipid reserves for flight (43), while solitary *S. gregaria* have poorer flight performance than gregarious *S. gregaria*, correlated with the strength of the adipokinetic response (44). Additionally, recent studies on courtship in *S. gregaria* have shown the pheromone phenylacetonitrile (PAN) to play a role in modulating the interactions between males competing for the same female. In gregarious locusts, PAN acts to conceal males already mounted on a female and, in so doing, reduces sperm competition and homosexual encounters. Solitary males do not produce PAN and tests indicate that much greater competitive interactions occur where solitary and gregarious males are attempting to copulate or guard a female, than when two gregarious males are involved (45). These two phase-dependent differences in behaviour are similar to the responses we observe in locusts exposed to fever temperatures in the current study. Together with the results of our earlier studies (17), this suggests that regular exposure to fever temperatures can uncouple the various gregarisation cues, causing a reversion towards the *solitaria* base state, even where gregarising stimuli from conspecifics are present.

The suggestion that fever affects locust phase state adds complexity to determining whether the fever-induced changes in life-history traits we observe in the current study represent costs of fever or not. On the one hand, even though poorer mate guarding and flight potential are

more subtle fitness responses than reductions in fecundity and survival, they might still represent significant ecological costs, especially if an individual is fevering amongst healthy conspecifics. Thus, if fever disrupts the synthesis or response to hormones, such as AKH, or pheromones, such as PAN (whether through an energetic trade-off or, for example, temperature sensitivity in the endocrine pathways), then this could be defined as a cost of fever. On the other hand, solitarisation is not just characterised by changes in flight and mate guarding. In the field, solitary locusts tend to disaggregate which will likely reduce density-dependent disease transmission risk and result in oviposition away from gregarious egg beds that could be foci of future infection (46). A shift towards the *solitaria* phase state, therefore, may actually represent a facultative change in life history that serves to limit the impact of disease moret 2003. Thus, rather than a cost, the life history changes we observe in the current study could be part of an additional defense response triggered by a fever signal. These contrasting hypotheses highlight the need for careful consideration in selecting appropriate life-history traits and interpreting fitness costs since these depend on both the mechanisms through which responses are mediated, and on the ecological context in which the interactions are played out. These results also highlight the potential complexity and subtlety of defense mechanisms and, we would suggest, underline the need to examine such phenomena in greater depth both from a mechanistic viewpoint and in ecologically meaningful contexts (c.f. 47).

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FIGURE LEGEND

Fitness correlates (means ± S.E.M. from five replicates) measured from adult desert locusts subjected to simulated fever regimes during sexual maturation. Locusts were kept at 38°C for 7 hours during the day; within this period, treated locusts were elevated to 42°C for 1 or 5 hours per day, on days 1-10, 11-20 or 1-20 of the maturation period. There were no significant differences in survival times of (a) females or (b) males (Kaplan Meier survival analysis with log-rank comparison of means) or (c) in female fecundity (by two-way ANOVA). Male mating competitiveness (d) (mate guarding against untreated males) was significantly different from zero in all treatments bar one and the control (sign tests, * is $P < 0.05$, ** is $P < 0.01$, *** is $P < 0.001$, n.s. = not significant). Male ability to sustain flight in a wind tunnel over 3.5 hrs. (e) was greater in controls than pooled treatments (F-test by deletion in a linear mixed effects model, differences at $P < 0.001$ denoted by letters) but there were no significant differences within the fever treatments.

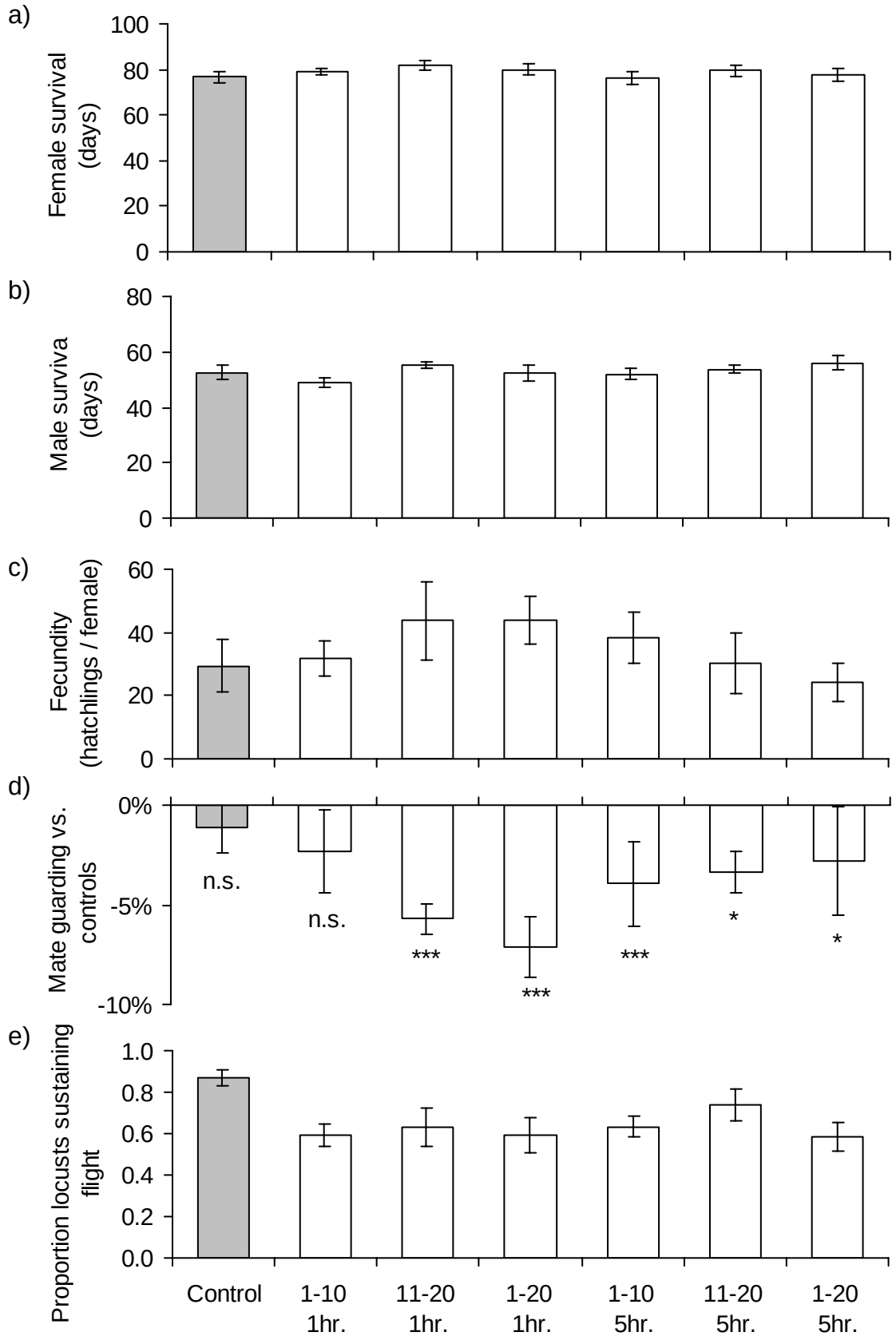
a)
Female survival (days)
100
80
60
40
20
0
b)
Male surviva (days)
80
60
40
20
0
c)
Fecundity (hatchlings / female)
60
40
20
0
d)
Mate guarding vs. controls
0%
-5%
-10%
n.s.
n.s.
***
***
***
*
*
e)
Proportion locusts sustaining flight
1.0
0.8
0.6
0.4
0.2
0.0
Control
1-10 1hr.
11-20 1hr.
1-20 1hr.
1-10 5hr.
11-20 5hr.
1-20 5hr.

FIGURE 1

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THE ENVIRONMENTAL IMPACT OF BIOLOGICAL AND CHEMICAL INTERVENTION FOR LOCUST CONTROL AGAINST NON-TARGET ARTHROPODS IN A RED LOCUST RECESSION AREA IN TANZANIA

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INTRODUCTION

Outbreaks of the red locust, *Nomadacris septemfasciata* Serville, are known to originate from nine relatively small and discrete source areas in Central and East Africa (Gunn, 1960; Bahana and Byaruhanga, 1991). These outbreak areas are typically treeless grassland plains or lake basins, associated with impeded drainage, which are prone to flooding during the rain season (Vesey-Fitzgerald, 1955). The population dynamics and ecology of the red locust has been well studied in the key Lake Rukwa outbreak area in Tanzania (Lea and Webb, 1939; Vesey-Fitzgerald, 1955; Symmons, 1959; Symmons and Carnegie, 1959; Dean, 1967; Stortenbeker, 1967).

Large-scale outbreaks of the red locust have the potential to threaten agricultural production throughout sub-saharan Africa, as occurred during the last major plague cycle between 1927-1945 (Morant, 1947). However, chemical control intervention over the past 50 years by the International Red Locust Control Organisation for Central and Southern Africa (IRLCO-CSA) has largely restricted gregarious locust populations to the remote outbreak areas and has succeeded in preventing the development of new plague cycles (Byaruhanga, 1999).

Management of the red locust currently relies on monitoring locust populations in the key outbreak areas, backed up by the aerial spraying of adult concentrations during the dry season before breeding occurs. However, during widespread population eruptions it is sometimes necessary to initiate chemical control early against gregarious hopper populations and fledglings within the flooded grasslands before they can form swarms and escape control. Once swarms emigrate from the outbreak areas they become very difficult to track down and control in the vast invasion area (Morant, 1947).

Chemical control of the red locust is currently undertaken by ULV aerial spraying with the organophosphate insecticide, fenitrothion (96% technical), applied at a volume application rate of 0.5ℓ/ha, giving a dose rate of ±500g a.i./ha. Fenitrothion is very effective against the red locust, giving >95% mortality with 24-48h. Although fenitrothion is relatively non-persistent in the environment, with a half-life of <3days, it is a broad-spectrum insecticide with high toxicity against almost all non-target aquatic and terrestrial arthropods (Thomas, Wood, Langewald and Lomer, 1997; Peveling, 2001).

Furthermore, if not handled and applied correctly it is known to be hazardous to spray operators, livestock and birds.

The seasonally flooded grasslands inhabited by the red locust provide a valuable ecological resource in the otherwise dry savanna areas of Central and Eastern Africa (Vesey Fitzgerald, 1955). Many of these grasslands also provide habitat for game animals and a wealth of bird life and are regarded as vulnerable habitats that are priority areas for conservation (Olson and Dinerstein, 1998; Peveling, 2001). The Kafue Flats in Zambia, for example, is recognised as the world's 4th largest refuge for bird life and has been designated a 'Ramsar site' of international conservation importance.

The aerial application of fenitrothion UL for red locust control during the height of the dry season, when the floodwater has receded and the grasslands are often devastated by fire, usually produces a comparatively low environmental impact (Bahana, pers comm.). However, the application of insecticide when biodiversity is high during the wet season or while the grasslands are still flooded, is a serious cause for concern (Peveling, 2001). More environmentally benign and sustainable methods of managing the red locust are urgently required.

The red locust has been considered a potential candidate for biological control with pathogenic microorganisms, as the high humidity within the grasslands during the wet season is likely to favour pathogen persistence and transmission. The first concerted attempt at bio-control of the red locust with entomopathogenic fungi dates back to the 1930s when hopper bands were sprayed with an aqueous suspension of spores of *Beauveria bassiana* Balsamo (Vuillemin) in Zululand in South Africa (Schaefer, 1936). However, the formulation of the aerial conidia of various isolates of the deuteromycete fungus, *Metarhizium anisopliae* var. *acridum*, in an oil carrier instead of water was found to greatly enhance the virulence of the fungus under conditions of low humidity, and opened the door for the development of effective myco-insecticides against locusts and grasshoppers (Bateman, Carey, Moore and Prior, 1993; Lomer and Prior, 1992). Oil-based myco-insecticides have produced effective control of a number of pest acridids in Africa and Australia under a range of environmental conditions (Lomer, Prior and

Kooyman, 1997). The myco-insecticide developed by the LUBILOSA programme (CABI Bioscience, Ascot, UK) was commercially registered against the brown locust, *Locustana pardalina* (Walker), in South Africa in 1998 as 'Green Muscle®'.

Red locust hoppers proved susceptible to Green Muscle® in laboratory bioassays and efficacy against gregarious hopper bands in the field was demonstrated in small-scale trials in the river Buzi floodplains in Mozambique (Price, Müller, Brown, D'Uamba and Jone, 1999). Participants at the DFID-funded Migrant Pests Workshop, held in Pretoria in 1999, identified the testing of pathogens and the development of alternative IPM strategies as priorities for locust control in Africa (Cheke, Rosenberg and Kieser, 2000). DIFID subsequently sponsored a project for the evaluation of Green Muscle® for the environmentally sustainable control of the red locust in its outbreak areas. A preliminary aerial spray trial of Green Muscle® against red locust hoppers was undertaken in the Kafue Flats in Zambia in February 2001. As part of the second season of field trials undertaken in Tanzania during 2002, a short-term study was undertaken to monitor the environmental impact of Green Muscle® against non-target arthropods. The results of this field work are now described.

MATERIALS and METHODS

Trial sites

Gregarious red locust hopper populations were located in the Iku-Katisunga grassland plains (06°56'S, 31°11'E) within the Katavi National Park in south-western Tanzania during February 2002. The Iku-Katavi has recently been one of the more active red locust outbreak areas and chemical control operations against adult populations have been necessary during the past few years (Bahana and Byaruhanga, 1999). The Iku-Katisunga plains measure approximately 28km north to south, with a mean width of 11km, and form part of the North Rukwa plains which stretch 140km south to the Rukwa valley and its shallow saline lake. The area receives an annual rainfall of 50-100cm,

most of which falls during a single summer wet season, from November to April. The winter season is usually very dry.

The lower areas of the Iku-Katavi plains are subject to occasional flooding by the Katuma river, which flows from lake Katavi and breaks through a sandy ridge at Sitaliki village to flow into the grasslands via a network of channels. Flood water and the accumulation of local rainfall causes water-logging of the grasslands. Soils in the floodplain grasslands are dominated by impervious black clays, which rapidly become waterlogged. However, under dry conditions the clay becomes hard and deeply fissured.

Vegetation in the Iku-Katisunga grasslands is dominate by a dense stand of *Echinochloa pyramidalis* (Vesey-Fitzgerald, 1955). Towards the perimeter of the plain, rain grassland species such as *Hyparrhenia* spp., *Sporobolus* spp. and *Cynodon dactylon* become more dominant. The interface between the grass plains and the *Combretum* spp. woodland was usually very sharp. The grasslands are occasionally subjected to extensive burning during the dry season, with uncontrolled fires being set by the local communities.

Locust surveys

Aerial surveys during September 2001 confirmed the presence of adult red locust concentrations in various parts of the Iku-Katisunga grasslands. Selected areas, previously marked with a GPS, were re-visited during helicopter surveys and foot safaris during February 2002 to determine the presence of hopper populations. Areas containing suitable hopper concentrations were marked with a GPS and the boundaries of two blocks of grassland, each approximately $1km^2$ (100ha) in extent, were marked with white plastic bags which were highly visible in the tall grassland. Hopper populations comprised mixed L4-L5, which predominantly exhibited a gregaria/transience coloration. In some areas the hoppers were coalescing into bands with hopper densities of up to $30/m^2$ recorded.

Myco-insecticide application

Green Muscle® OF formulation (Biological Control Products (Pty), Ltd. (BCP), South Africa), was diluted in pure grade paraffin (Jet A1) on site at Mpanda airfield to give a

calculated spore dose rate of 50g/litre. The myco-insecticide was applied through two Micronair AU4000 atomisers attached under the wings of the IRLCO-CSA Cessna 185 spray aircraft. The VRU of the atomisers were set to No.9 position to give the required flow rate of 11ℓ/min and the Miconair blade angle was set at 45° to provide a coarse droplet size (±100µm) suitable for impaction upon vegetation. Application was undertaken on 14 February 2002 at an air temperature of 26-28°C and with a variable breeze of 2-5m/s blowing from between SW and W/NW. The aircraft flew spray runs from NW to SE, flying at 90mph (145km/h) with a track spacing of 50m and an emission height of 10-15m to give a volume application rate of 2ℓ/ha. Track interval was marked by flagmen on either side of the spray plot who guided the spray runs via radio contact with the pilot.

Fenitrothion toxic standard

An aerial application of fenitrothion was undertaken on the morning of 18 February. The standard volume application rate of 0.5ℓ/ha (96% fenitrothion UL) was achieved by setting the VRU to No.7, giving a dose rate of ±500g a.i./ha. All other aircraft application parameters were the same as during the Green Muscle® application. Metereological conditions were clear, with a slight breeze of 0-1m/s blowing from the SW. The aircraft flew spray runs from NW-SE.

Paired control plots

The plot selected for the Green Muscle® trial was situated ±3km out into the plains and was virtually all flooded with water, which varied in depth from 2-50cm. A few large mounds of the termite, *Macrotermes vitrialatus* (Sjöstedt), dotted within the plot provided the only dry ground. Vegetation mainly comprised a uniform stand of 2-3m high *E. pyridaminalis* grass and the soil surface was mainly bare of grass litter due to burning the previous season. In contrast, the fenitrothion plot, situated 3-4km away and close to the tree line, was completely dry and was vegetated with a 1-2m high mixture of grasses. Game animals, especially elephant and buffalo, were regular visitors within the plot. The soil surface here was covered with a dense layer of dry grass, indicating that no burning had occurred during the previous dry season. No termitaria were present.

Since conditions within the two plots were obviously different, making direct comparisons impossible, it was necessary to mark out an untreated control plot in a similar habitat near each of the treatment plots. The paired control plots were approximately 500m upwind of their respective treated plots, which allowed a timeous and more direct comparison of the impact of the insecticide treatments compared with the controls.

Spray deposition in a grassland habitat

During aerial spraying over a tall-grass habitat, the majority of spray droplets are known to impact upon vegetation in the upper and middle canopy, with few droplets able to penetrate down to the soil surface. Likewise in the present study, the filtering effect of the grass was clearly demonstrated during application of a diesel-oil spray applied at 1ℓ/ha from a hand-held Micron Ulva+ sprayer (Micronair, Bromyard, UK). Visual examination of oil sensitive papers (Teejet Spraying Systems, Wheaton, USA) set out at different heights from 0-2m in the grassland, revealed that most diesel spray impacted vegetation in the upper canopy with only the smallest droplets finding their way to the soil surface. Following this preliminary demonstration it was decided to concentrate observations on non-target invertebrates inhabiting the middle to upper vegetation strata, as this is where the initial impact of the insecticide treatments was likely to be greatest.

Sampling of non-target invertebrates

There is still considerable debate regarding the most effective methods of sampling invertebrates in environmental monitoring programmes, as different sampling methods selectively sample fauna depending upon the environmental conditions. The flooded, tall-grass habitat in the Iku-Katisunga plains posed a challenge for the sequential sampling of invertebrates. Sweeping the vegetation with a sweep net was considered the most effective method of sampling, especially with the time constraints associated with helicopter transport to the plains. Sweeping is also recommended for invertebrate sampling in grasslands (Southwood, 1978). As a back-up to sweep-netting, timed visual count sampling of selected invertebrates was also undertaken.

The invertebrates inhabiting the grass canopy were observed to migrate up and down the grass during the day, moving up into the canopy during late morning after the heavy dew had dissipated. They subsequently descended during the heat of the day and moved back up into the canopy during the late afternoon. No observations were made at night. In order to standardize catching efficiency, it was important to undertake sampling at approximately the same time each day. Sampling thus only undertaken between 10h30-13h00 once the grass had dried off and invertebrates were visible and active.

Pre-spray surveys for indicator species

Surveys were undertaken within the marked plots a few days before spray application to evaluate the diversity of non-target invertebrates and to select suitable indicator species for assessment during the post-application monitoring period. Species selected were distributed throughout all trial plots, were easily recognisable and were readily captured in sufficient numbers in the sweep net to allow statistical analysis to be undertaken.

Sweep net sampling

Individual sampling events consisted of making five strong sweeps with a 50cm diameter canvas net through the grass, covering an 180° arc in front of the body at waist height, while walking slowly through the grass. After five individual sweeps the net was folded over and the contents shaken to the bottom of the net where the catch was examined. The numbers of each indicator species were counted and recorded in a notebook. The entire contents of the net were then released unharmed back into the grass. After walking a further 15-20 paces through the grassland the five-sweep process was repeated. A total of 50 replicated sampling events were undertaken along diagonals through each plot during each visit. Sampling within each plot took approximately 1.5 hours to complete.

Due to the limited access to the grasslands via helicopter, sweep sampling could not be undertaken as often as desired. Sampling in the Green Muscle® plot and its paired control was undertaken the day before application (pre-survey) and then at 3, 6, 9, 12 and 15 days post-application. Sampling in the fenitrothion plot and its paired control was undertaken the day before application and then at 1, 3, and 9 days post-application.

During each visit to the Green Muscle® plot and its paired control a selection of non-target invertebrates were collected and placed into plastic 1ℓ bottles, sealed with nylon gauze, and maintained in a makeshift laboratory at the Mpanda hotel for up to four weeks post-application. Grass-feeding beetles, spittle-bugs and grasshoppers were selected as these insects could be maintained for an extended period under these artificial conditions. Fresh grass was provided every 2-3 days. Any cadavers were removed and subsequently checked for signs of *Metarhizium* sporulation by incubating them under high humidity in glass tubes containing plugs of wet cotton wool.

Visual count sampling

Visual counts of selected longhorn beetle and dragonfly species were undertaken along transects walked through the spray plots. At intervals of 25 paces the observer stopped and waited for 30 seconds, until the disturbance caused by walking through the grass subsided, and then counted the number of longhorn beetles within an 180° arc at a distance of 1-2m from the observer. Dragonflies (both flying and settled) were counted in a similar manner at a distance of 3-4m from the observer. At each stop a further 30 seconds was spent searching for ants amongst the base of the grass stems.

Statistical analyses of the mean number of each indicator species captured during 50 sweeps, as well as those noted during timed counts in the treated and paired control plots on each sampling occasion, were undertaken using a student's t-test (GenStat, 2000).

Pitfall traps are known to be an effective method for evaluating species richness and population densities of ground-dwelling beetles, spiders and ants (Southwood, 1978). It was initially intended to undertake an extensive pitfall trapping campaign in the Iku-Katisunga grasslands, but this proved impractical due to the flood water in the Green Muscle® plot and the impenetrable clay soil conditions in the fenitrothion plot. However, a limited network of 12 traps were set out around the base of termite mounds in the Green Muscle® plot and its control, as this was the only unflooded area available. Traps consisted of two plastic drinking cups, 5cm in diameter, inserted one inside the other and placed into a hole dug in the soil. The top of the trap was made flush with the soil surface. The inner cup was partly filled with water, which was then super-saturated

with salt as a temporary preservative. A few drops of house-hold washing-up liquid was added to the salt water to reduce the surface tension and allow trap catches to sink. Catches were removed every three days by removing the inner cup of the trap. Specimens were placed in tubes of 70% alcohol and the traps were then re-set.

Ecological processes

Scavenging

The effect of spray application on ecological processes was briefly investigated by comparing scavenging activity by invertebrates of pieces of a local oily-bread delicacy, known as 'half cakes', placed out on the soil surface. In the Green Muscle® plot and its control, blocks of 'half cake', measuring 3x3x3cm, were placed at four sites around the base of mounds (n=5) of the termite, *M. vitrialatus*, as this was the only dry ground available. The location of the cakes were marked with white plastic bags tied in the grass. No termite mounds were present in the fenitrothion plot and its control, so similar blocks of cake (n=20) were laid out at 25m intervals along transects. The blocks of 'half-cake' were examined after three days and the proportion consumed assessed visually on a scale of 0 to 5, where 0 represented no consumption and 5 total consumption. A fresh block of cake was then set out at each site for a further three days.

Decomposition

In an attempt to evaluate the effect of insecticide application on microbial activity and its possible impact on the biological process of decomposition, a simple experiment to evaluate the decomposition of filter paper discs was undertaken in the grasslands. Twenty filter paper discs (9cm diameter) were pegged with wire directly to the soil surface along a transect through each trial plot. After 15 days exposure, the filter paper discs were removed, carefully washed in water to remove excess soil and placed in 80% alcohol. Back in the laboratory they were oven dried at 85°C for 6 hours and weighed. Twenty unused filter papers were similarly dried and weighed as controls.

RESULTS

Efficacy of Green Muscle against red locust hoppers

Following the aerial application of Green Muscle®, the mean survival time of treated hoppers, collected in the field 1d post-application and maintained in plastic bottles, was 20±1d compared with 36±2d for untreated controls maintained under identical conditions. A log rank comparison of the treated vs untreated survival curves gave a significant difference at $P<0.0025$ (Elliot, 2002). Decline in locust numbers in the field was difficult to demonstrate due to movements of the hopper bands during the trial period. However, after 15 days there was high mortality within the treated plot and the population was estimated to have declined by 90% compared with the untreated control (Elliot, 2000).

The speed of mortality of treated hoppers in the field, and especially those maintained in the plastic bottles, was substantially slower than expected from previous trials with Green Muscle®. This indicated that there had been problems with either the viability of the spore formulation, the spray application itself or with the secondary pickup of spore residues by locusts from the treated vegetation.

Spore viability

Samples of Green Muscle® myco-insecticide collected from the aircraft spray tank after application, stored in glass tubes and examined for viability three weeks later in the laboratory, showed no detectable spore germination on agar plates. Although spore viability is known to decline after formulation in paraffin oil, additional factors are thought to have accentuated the loss of spore viability. The exposure of the spore concentrate to excessive heat conditions during transport through Tanzania, along with the comparatively high water content of the original spore concentrate (10% versus a recommended maximum of 5% water), may have played a role (Elliot, 2002). These various factors suggested that the locusts received a very low dose of viable spores during the current application, which is backed up by evidence from the slow speed of kill of treated hoppers.

Aerial application

The aerial application of Green Muscle® was inconsistent due to the variable wind speed and direction, which caused uneven spray deposition and 'striping' in some areas of the spray plot. However, this was considered relatively unimportant in the current trial as it is known that the prime method of dose accumulation of Green Muscle® spores by mobile acridids in a grassland habitat is by secondary pick-up of spore residue from treated vegetation (Thomas, Langewald and Wood, 1997).

There were also some problems with the aerial application of the fenitrothion, with certain patches of grassland receiving little or no insecticide. However, fenitrothion application over most of the spray plot was considered effective, despite the difficult application conditions within the grasslands.

Sweep-net sampling

Sampling for invertebrates was very effective after 10h30 in the morning, once the heavy dew on the grass had dried off. Although meteorological conditions of cloud cover, air temperature and wind speed varied between sampling events, on no occasion were conditions too adverse for effective sweep sampling to be undertaken.

The diversity of invertebrates within the grass plains was surprisingly poor, compared with the rich bio-diversity observed in the woodlands bordering the plains. However, certain invertebrate species were relatively abundant and conspicuous in the middle to upper grass strata throughout the grasslands, which made them suitable indicator species for the purposes of environmental monitoring.

Invertebrates selected as indicator species for the post-application assessments were 3 species of short-horned grasshoppers, 1 long-horned grasshopper, 2 beetles (longhorn beetle and leaf beetle), 1 bug (leaf hopper), 1 spider and 1 tick species (Table 1).

Table 1. Invertebrates selected as indicator species in the grass canopy

Orthoptera
- Acrididae
  - *Orthoctha* sp.
  - *Euprocnemis sp.*
  - *Mesopis* sp.
- Tettigoniidae

Coeloptera
- Cerambycidae
  - Lamiinae
    - (Gen. et spec. indet.
- Chrysomelidae
  - Galerucinae
    - Cf. *Monolepta* sp.

Homoptera
- Cercopoidea
  - Aphrophoridae
    - *Clovia* sp.

Arenae

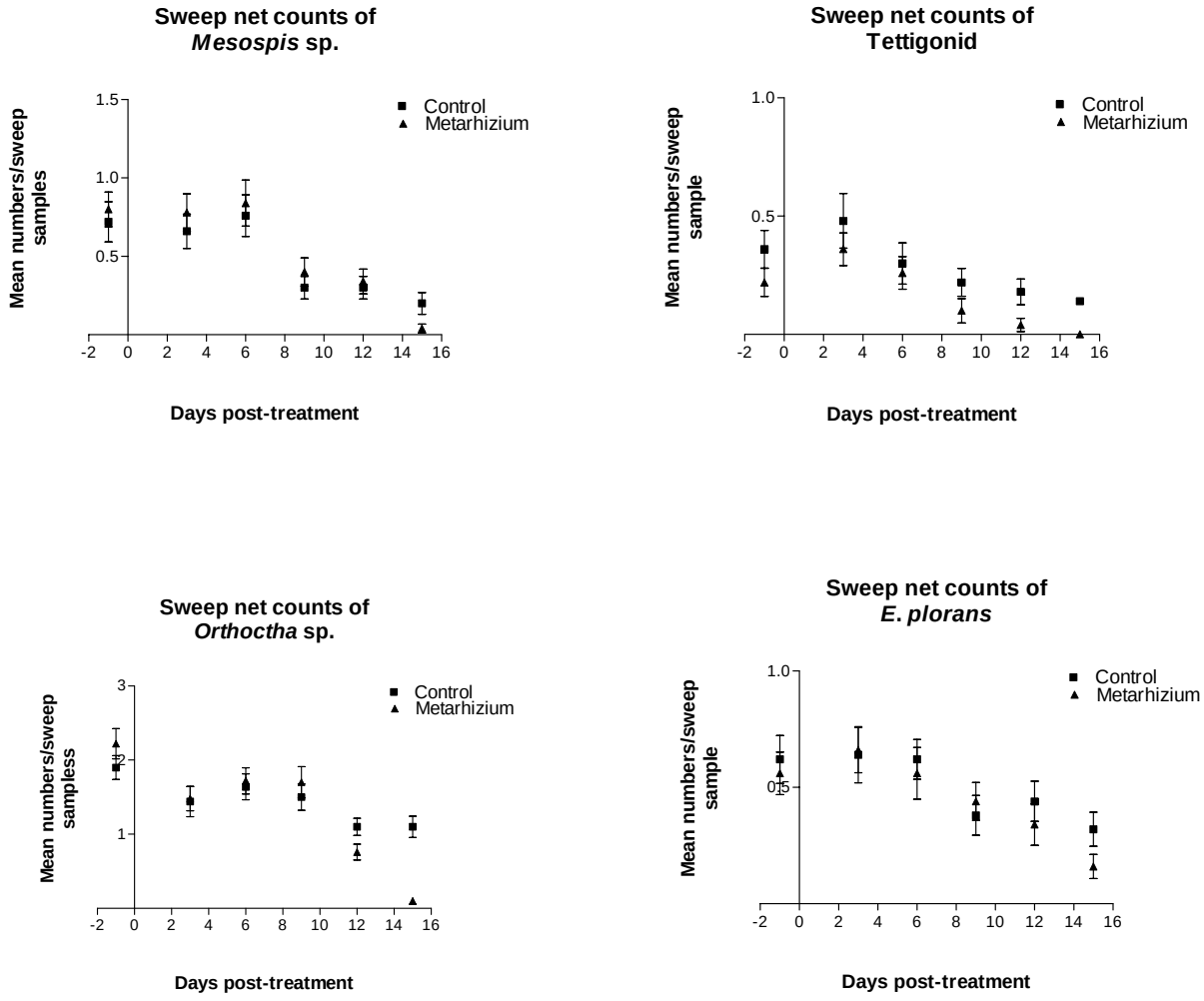
Tick

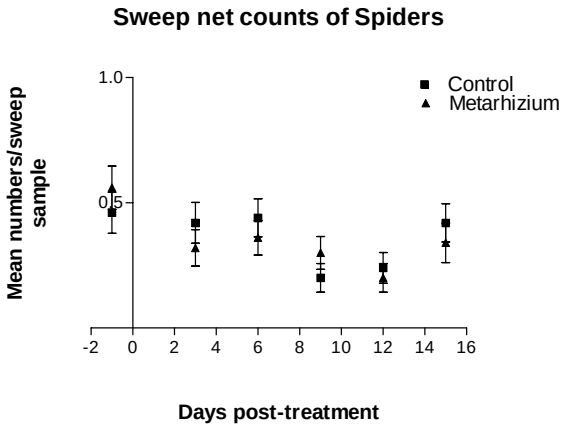
The above tick species was commonly distributed throughout the fenitrothion plot and its control, but was rarely found in the flooded Green Muscle® plot and its control, making it an unsuitable indicator species in these latter plots. Likewise, the *Mesopis* sp. grasshopper was rarely encountered during sweeps in the dry fenitrothion plot and the paired control, while it was numerous in the flooded Green Muscle® plots.

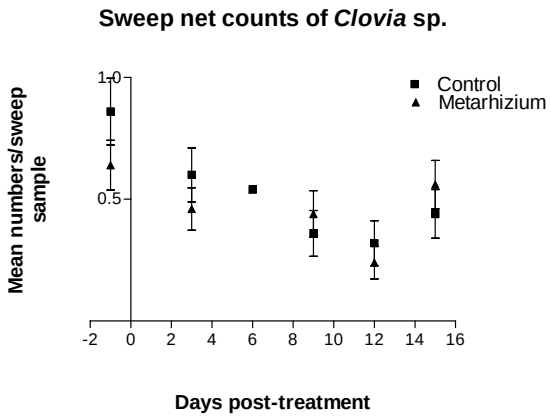
Green muscle plot and paired control

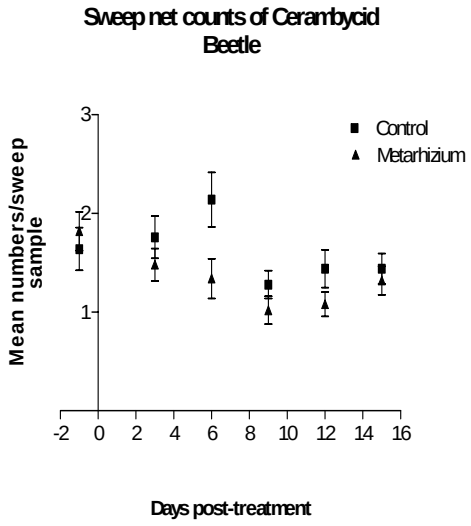
The mean number of each indicator species, captured during 50 sweep-net samples undertaken in the Green Muscle® plot and its paired control at each sampling date, are depicted in Fig. 1.

Fig. 1. Mean numbers of each indicator species collected during 50 sweep net samples undertaken in the *Metarhizium* spray plot and the untreated control during different sampling events.









Statistical analysis, using the Student's t-test, was undertaken to compare the mean counts of each indicator species collected during 50 sweep net samples in the Green Muscle® treated plot and the untreated control at each sampling date. T-statistic values obtained are given in Table 2.

Table 2. t-statistic values calculated from the comparison of mean counts of each indicator species at each sampling date in the Green Muscle® plot and its control. All significantly different 't' values, at probability levels $p \leq 0.05$ (*) and $p \leq 0.01$ (**), are indicated.

	Date of sweep-net sampling					
Indicator species	-1 day	+3 days	+6 days	+9 days	+12 days	+15 days
Orthoctha sp.	-1.23	-0.15	-0.32	0.73	2.18*	6.67**
E. plorans	0.44	-0.13	0.43	-0.51	0.81	1.79*
Mesopis sp.	-0.47	-0.74	-0.40	-0.87	-0.38	2.12*
Tettigonid	1.41	0.90	0.36	1.53	2.27*	3.81**
Cerambycid	-0.62	1.04	1.34	1.31	1.57	0.57
Chrysomelid	-0.74	0.91	-0.99	-0.87	-0.96	-1.14
Clovia sp.	1.29	1.00	0.67	-0.60	0.70	-0.85
Spider	-0.84	0.92	0.78	-1.15	0.48	0.73

Following aerial application of Green Muscle®, no unusual behavior or mortality of any of the indicator invertebrates was observed in the treated plot or the control for up to 12d post-application. However, between 12-15d post-application, there was a statistically significant drop in the numbers of each of the grasshopper indicator species in the treated plot compared with counts in the untreated control (Table 2). In contrast, counts of the indicator beetles, bug and spider did not differ significantly throughout the 15d post-application monitoring period.

Intensive searches of the soil surface and surface water, undertaken on hands and knees along transects through the Green muscle® spray plot on day 12 and 15 post-application, revealed the remains of a limited number of cadavers of various grasshoppers and red locust hoppers. Such cadavers were often tinged red in colour, which is a positive indicator of *Metarhizium* mycosis. Scavenging of these cadavers by ants and beetles was readily observed. A number of sick and dying grasshoppers and locust hoppers were also observed and these sluggish individuals made easy targets for predation by birds, ants, beetles and frogs. No sick or dead grasshoppers or locust were observed during similar surveys of the soil surface within the untreated control plot.

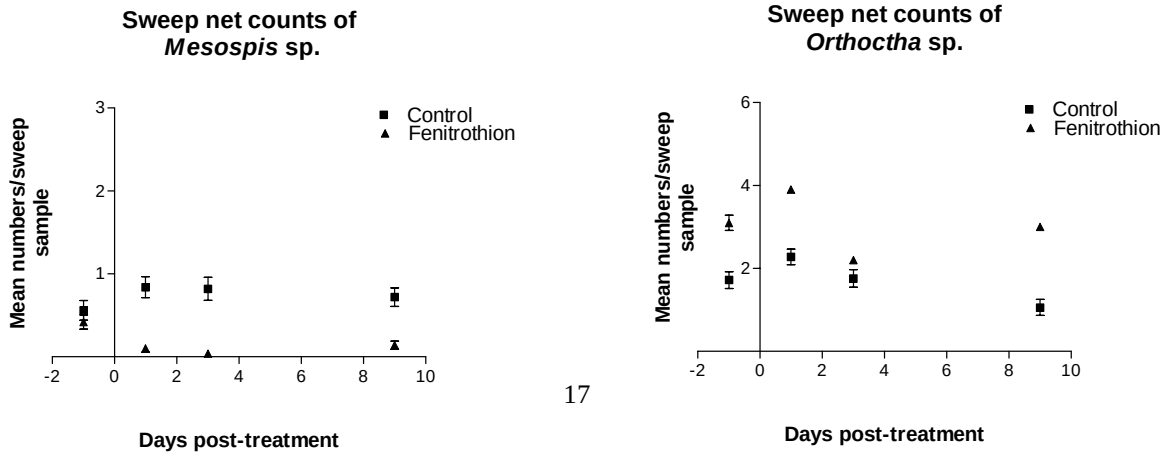
Samples of grasshoppers, collected within the Green Muscle® plot at various times post-application and subsequently maintained in plastic bottles, all died within 10-15 days. Some of these cadavers turned a red colour, indicating mycosis, and some produced green *Metarhizium* fungal outgrowths a few days later. However, most cadavers rapidly decayed under the humid conditions before mycosis could be verified. Although some grasshoppers captured in the untreated control plot and maintained in bottles also died within 15 days, none developed signs of mycosis or fungal sporulation.

None of the other non-target invertebrates maintained in plastic bottles for up to 28d post-application, showed any sign of mycosis or fungal sporulation.

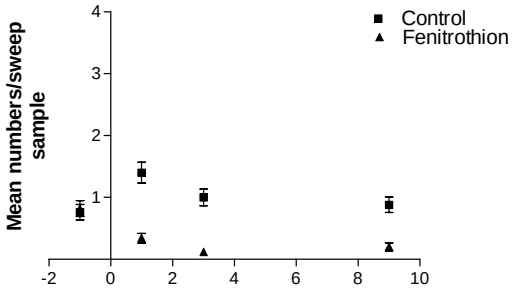
Fenitrothion plot

Following aerial application of fenitrothion 96UL, the first toxic effects against non-target invertebrates were observed within 3-4 hours, when grasshoppers and beetles started to fall from the grass canopy onto the soil. Within 24h there had been a dramatic collapse of all invertebrate populations inhabiting the grass canopy and a range of dead and dying invertebrates were found on the soil surface. The statistically significant decline in the number of indicator species in the fenitrothion plot compared with the untreated control is shown in Fig. 2. Statistical data are provided in Table 3. Within 3d post-application, virtually no invertebrates were present in the insecticide-treated grass canopy.

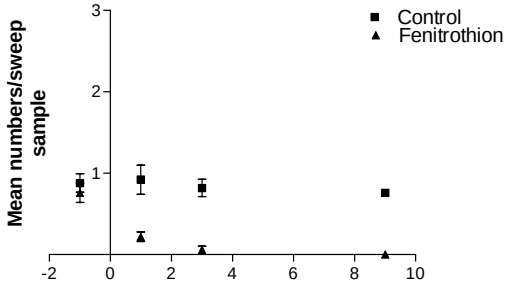
Fig. 2: Mean numbers of each indicator species collected during 50 sweep net samples undertaken in the fenitrothion spray plot and the untreated control during different sampling events.



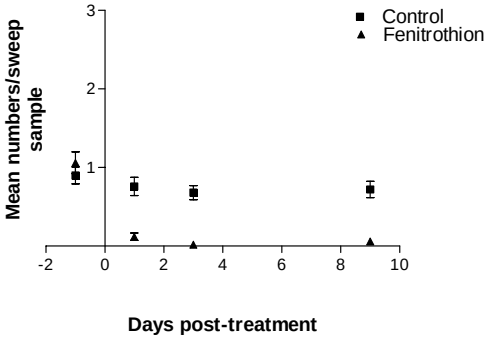
Sweep net counts of *E. plorans*



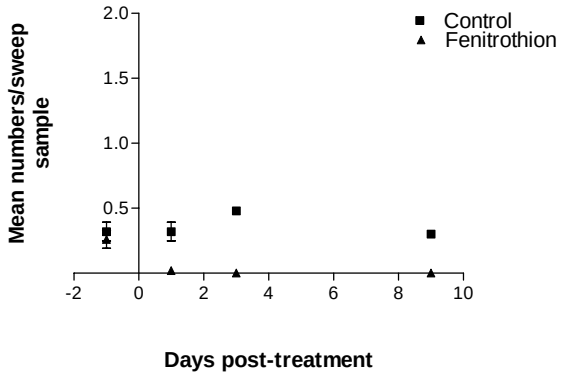
Sweep net counts of Cerambycid Beetle



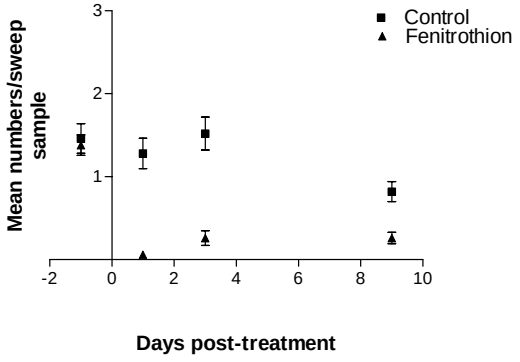
Sweep net counts of *Clovia* sp.



Sweep net counts of Spiders



Sweep net counts of Beetle2



Sweep net counts of Ticks

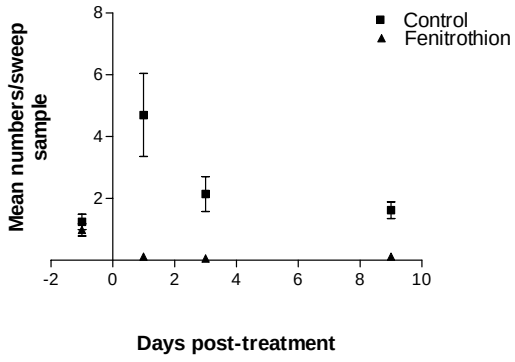


Table 3. t-statistic values calculated from the comparison of mean counts of each indicator species at each sampling date in the fenitrothion plot and the untreated control. All significantly different 't' values, at probability levels (under the null hypothesis) of $p \leq 0.05$ (*) and $p \leq 0.01$ (**), are indicated.

	Date of sweep-net sampling					
Indicator species	-1 day	+1 day	+3 days	+9 days		
Orthoctha sp.	1.34	10.53**	7.57**	8.57**		
E. plorans	-0.34	5.68**	6.20**	4.90**		
Mesopis sp.	0.96	5.57**	5.50**	4.78**		
Cerambycid	0.73	3.73**	6.63**	5.21**		
Chrysomelid	0.37	6.55**	5.79**	4.04**		
Clovia sp.	-0.91	5.11**	7.33**	6.08**		
Spider	0.60	3.99**	5.66**	5.98**		
Tick	0.82	3.40**	3.67**	5.46**		

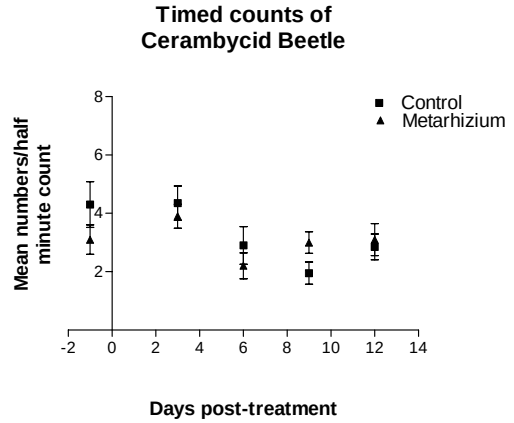
Although there was a statistically significant reduction in numbers of all indicator species in the treated grass canopy for at least 9 days post-application, the more mobile winged grasshopper and beetle species showed the first signs of re-clonisation of the treated plot at the 9d count (Fig. 2).

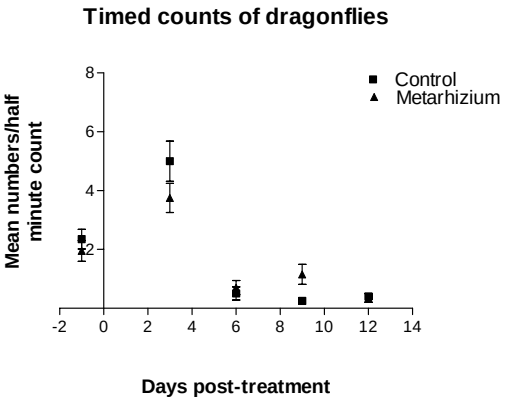
A number of dead ants and beetles were found on the soil surface within 1-3 days after treatment with fenitrothion. However, other ants and beetles were regularly observed scavenging the cadavers of invertebrates that had been killed by the insecticide. This observation indicated that there was undoubtedly less direct negative impact on geophile fauna compared with grass canopy fauna.

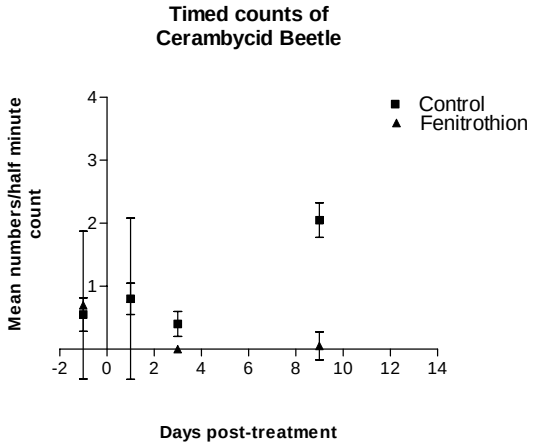
Visual Insect counts

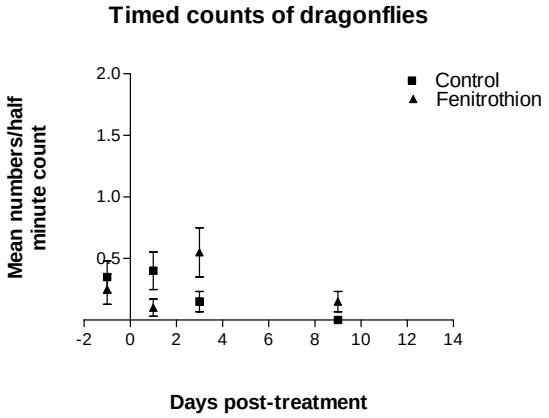
The numbers of the longhorn beetle species and dragonflies noted during timed counts undertaken on each sampling date in the Green Muscle and fenitrothion spray plots and their paired controls, are depicted in Fig.3.

Fig. 3. Timed counts of indicator species in the Green Muscle and fenitrothion plots and their paired controls.









The grey-coloured, long-horn beetle species was very common throughout the grass plains, especially in areas of tall *E. pyramidalis* grass. Upon being disturbed, these beetles either hid behind the grass stem, ran up or down the stems or dropped to the ground and "played dead", but never did they fly. The counts of beetles did not differ significantly between the *Metarhizium* treatment and the control plot, which confirmed the sweep net data. In contrast, beetle numbers dropped to zero within three days following fenitrothion application. The anomaly of the number of beetles found alive in the treated plot 24 hours post treatment was due to the stripy aerial application of the insecticide.

Dragonfly numbers and behaviour were unaffected by the Green Muscle® application. After an initial drop in dragonfly numbers in the fenitrothion plot, numbers of these mobile insects started to recover in the treated plot after 3 days post-application.

Ecological processes

Scavenging

The half-cakes technique initially worked well and the cakes were readily scavenged by ants and ground beetles. However, the white plastic bag markers tied in the grass attracted the attention of elephants and topi antelope, which then consumed the delicacies soon after they had been laid out. The animals continued to search for the cakes even after the marker bags had been removed. This confounded the results and the experiment had to be abandoned.

Decomposition

The filter paper discs were exposed in the field for too short a time (12 days) to demonstrate any changes in the rate of decomposition. In fact, the papers provided an ideal growth medium for fungi and most actually gained weight during the experiment. Others were partially eaten by snails and beetles. For more reliable results in future, the filter papers should be confined within exclusion nets for an extended period.

Pitfall traps

The series of 12 pitfall traps set out in the Green Muscle® plot and its control succeeded in capturing a variety of invertebrates, of which ants, ground beetles and spiders were the most common (Table 4). A number of snails, earthworms and frogs were also captured. Unfortunately, the numbers captured per 3d period in both plots were low and did not allow statistical analysis to be undertaken. However, there were no obvious trends in the numbers or types of organisms captured in the traps during the trial period (Table 4).

Table 4. Total number of organisms captured in a series of 12 pitfall traps in the Green Muscle® (GM) plot and untreated control per 3 day sampling period post-application.

	3-6 days p.a.		6-9 days p.a.		9-12 days p.a.		12-15 days p.a.	
	GM	Control	GM	Control	GM	Control	GM	Control
Coleoptera	8	7	3	16	14	4	7	4
Orthoptera	5	1	2	4	3	6	1	5
Diptera	5	2	3	1	3	8	6	5
Hemiptera	4	2	1	0	2	1	2	6
Hymenoptera – Ants	7	13	17	4	15	29	6	16
Hymenoptera – others	0	0	0	0	1	1	1	2
Blatoidea	0	1	1	0	0	0	0	1
Aranena	8	12	4	17	19	17	20	13
Harvestmen	2	2	0	7	0	1	0	1
Annelida	0	1	2	5	4	0	0	1
Gastropoda	2	1	0	0	2	0	0	1
Frogs	1	4	2	3	6	12	8	7

DISCUSSION

The study undertaken in the Iku-Katisunga grasslands in SW Tanzania demonstrated that an aerial application of Green Muscle® myco-insecticide could successfully control red locust hopper populations, while at the same time producing far less environmental impact than a conventional application of fenitrothion UL. With the exception of the grasshopper fauna, which proved susceptible to Green Muscle®, no direct mortality or other negative effects on non-target organisms were detected during the 15d post-application observation period. Similarly, non-target beetles and bugs collected in the Green Muscle® spray plot and maintained in plastic bottles, showed no signs of mycosis during a 4 week observation period. Unfortunately, no studies on long-term sub-lethal effects were possible during the trial period. However, the results confirmed previous environmental impact studies undertaken in the Sahel, which showed that apart from the susceptible grasshopper fauna, there was minimal impact of Green Muscle® on non-target organisms (Peveling, 2001).

In contrast, the toxic standard insecticide, fenitrothion 500g a.i./ha, had a devastating direct effect on virtually all non-target invertebrates inhabiting the middle to upper grass

canopy. Almost all invertebrates were completely eliminated from the grass canopy within 24-72h post-application. However, there was less direct toxic effect on some of the geophile ant and beetle fauna, which probably survived by being shielded by the dense grass from the direct impact of the fenitrothion.

Although fenitrothion is known to be an effective broad-spectrum insecticide, with a high direct impact on non-target invertebrates (Peveling, 2001), it is also known to have a short toxic half-life under tropical conditions. In the current study the more mobile grasshoppers, flies, wasps and dragonflies had started to re-colonise the fenitrothion spray plot within 9 days post-application. This re-colonisation process would probably have continued, but long-term observations could not be undertaken in the present study.

The minimal impact of Green Muscle® against non-target organisms in the red locust grasslands, compared with conventional insecticides, supports the findings of a number of previous impact studies (Peveling, 2001). The FAO pesticide referee group has already recommended *Metarhizium* myco-insecticides for acridid control in environmentally sensitive areas (FAO, 2000). The operational adoption of the Green Muscle® technology by IRLO-CSA is therefore advocated as a more environmentally benign alternative to fenitrothion spraying, especially during the wet season when biodiversity is greatest.

The negative impact of Green Muscle® on non-target grasshoppers is, however, a cause for concern. Grasshoppers are a vital component of the ecology of tropical grassland ecosystems and play important roles as herbivores and in the re-cycling of nutrients and energy flow systems (Gandar, 1982; Samways, 1997). The decimation of grasshopper populations from large areas of grassland following broad-acre application of most conventional insecticides, as well as Green Muscle®, is therefore very detrimental to the functioning of the grassland ecosystem. However, the targeted application of Green Muscle® to high-density red locust populations, allowing refuge areas for grasshopper populations between the treated areas, would likely have no long-term impact on grasshopper populations or the ecology of the grasslands.

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2.0 BODY TEMPERATURE MODELS OF LOCUSTS AND GRASSHOPPERS AND POSSIBLE IMPLICATIONS FOR BIOLOGICAL CONTROL

ABSTRACT

1. Biological control agents composed of entomopathogenic fungi such as *Metarhizium anisopliae* var. *acridum* and *Beauveria bassiana* are increasingly being used to control locusts and grasshoppers in different regions of the world. Efficacy of these pathogens is mainly limited by temperature. Many locusts and grasshoppers are able to maintain body temperatures at a "preferred" level within a narrow temperature range, often exceeding the maximal growth limits of these pathogens and so affecting pathogen performance.
2. In this study thermal behaviour of 11 acridid species (seven economically important pests requiring annual control and four of non-pest status that co-occur with target species), from different habitats and regions of the world, were captured through the development of species-specific body temperature models. Species-specific body temperatures models successfully described body temperatures for linear and non-linear methods ($R^2 > 0.70$) using a single variable, ambient temperature, for all species
3. Body temperature models between species were examined to determine whether a generic model can be derived to describe a range of species (particularly those of economic importance and which are subject to biocontrol operations). Analyses indicated that a generic body temperature model could be used to describe 3 economic species from South Africa, Spain and Australia. Accuracy assessments indicated that this model could predict body temperatures to within 1°C of species-specific models.
4. The generic model can be used to describe body temperatures for other economically important species (such as *Schistocerca gregaria* and *Locusta migratoria*) where body temperature models are missing but are known to have the same set point temperatures as *Locustana pardalina*, *Chortoicetes terminifera* and *Dociostaurus maroccanus*.
5. Body temperature models were used to explore possible efficacy of two different fungal-based biocontrol agents used to control locusts and grasshoppers in the field.

2.1 Introduction

A number of studies have demonstrated the importance of temperature and thermal biology in the outcome of host-parasite/pathogen interactions (for an overview see Thomas & Blanford, 2003). Recent work on the use of fungal pathogens for biocontrol of locusts and grasshoppers, for example, has shown that the ability of a pathogen to kill the host depends crucially on host body temperature and how this fluctuates with external environmental conditions. The speed of kill and overall mortality caused by entomopathogenic fungi, such as *Metarhizium anisopliae* var. *acridum* (= *Metarhizium flavoviride* Gams and Rozsypal, Driver *et al.*, 2000) and *Beauveria bassiana* (Balsamo) Vuillemin, vary greatly with changes in environmental temperature and host thermal biology (Inglis *et al.*, 1996, 1997; Blanford & Thomas, 1999a, b, 2000; Arthurs & Thomas, 2000; Elliot *et al.*, 2002; Ouedraogo *et al.*, 2003). The effect is that these pathogens might appear either very virulent, or virtually benign, depending on the ecological context (Blanford & Thomas, 1999b). For biocontrol, this means that biopesticide products based on these fungi will achieve excellent control under some conditions, whilst under other conditions they might be largely ineffective and inappropriate for use (Lomer *et al.* 2001).

Many locusts and grasshoppers are active behavioural thermoregulators. That is, via a combination of habitat choice and body orientation, these insects seek to achieve and maintain a preferred, or set point, body temperature over a range of environmental conditions (Chappell & Whitman, 1990) for as long as possible (see Stower & Griffiths, 1966; Lactin & Johnson, 1996b, 1998). The ability to achieve preferred temperatures is affected by an insect's colour (Forsman, 2000; Pepper & Hastings, 1952; Lactin & Johnson, 1998, 1996a,b), age, size and sex (Stower & Griffiths, 1966; Kemp, 1986), and is modulated by abiotic variables, such as wind speed, solar radiation, air temperature (Digby, 1955; Carruthers *et al.*, 1992; Blanford *et al.*, 1998; Blanford & Thomas, 2000), together with other biotic factors, such as vegetation cover (Anderson *et al.* 1979; Blanford & Thomas, 2000). More generally, ectotherm organisms can only maintain a preferred temperature when external conditions are conducive. Hence, at night, for example, body temperatures will tend to reflect more closely the ambient thermal fluctuations. In addition, for species that may occur in a range of habitats (e.g. in both long and short grass systems), or where conditions change over the course of the insect's lifetime (e.g. wet season to dry

season) the ability to reach preferred body temperature may vary over time and space (Huey, 1974).

The preferred body temperature selected is a compromise based on enzyme kinetics (Heinrich, 1977; Sharpe & DeMichele, 1977; Begon, 1983) aimed at optimising performance. In the context of an interaction with a pathogen, this translates to optimising immune responses to combat infection (Ouedraogo *et al.,* 2003; Thomas & Blanford, 2003). For many locusts and grasshoppers (particularly those from semi-arid regions) thermoregulatory behaviour enables these insects to raise their temperatures to a preferred set point around 38^{o}C-40^{o}C (Chappell & Whitman, 1990; Carruthers *et al.,* 1992; Inglis *et al.,* 1997; Blanford & Thomas, 1999a,b; Blanford *et al.,* 1998). Given that the optimum temperature for growth of fungi such as *B. bassiana* and *M. anisopliae* is in the range of 25^{o}C-30^{o}C (Fargues *et al.* 1997; Thomas & Jenkins, 1997), such body temperatures will also have a direct effect on pathogen growth, contributing to the substantial delay in the rate of fungus-induced mortality often observed (Kemp, 1986; Carruthers *et al.,* 1992; Inglis *et al.,* 1997; Thomas & Blanford, 2003). Not all grasshoppers and locusts are active thermal regulators (Anderson *et al.,* 1979; Blanford *et al.,* 2000), however, and even for some species that do thermoregulate, environmental conditions may preclude the attainment and/or maintenance of high body temperature (Begon, 1983). However, an inability to thermoregulate does not equate to a lack of thermal sensitivity. Thus, environmentally driven variation in body temperature will still influence key elements of the host immune system and affect pathogen growth. In addition, even in the absence of effective thermoregulation, body temperatures may show a marked non-linearity with ambient temperatures (Thomas & Blanford, 2003).

In sum, the body temperature of locusts and grasshoppers is dependent on environmental temperature, with the exact relationship determined by a number of ecological factors, particularly thermoregulatory behaviour. In turn, host body temperature determines the performance of key biocontrol agents such as fungal pathogens, either directly or through effects on host defence mechanisms. If we are to fully understand and predict variability in pathogen performance (which is essential in developing effective biocontrol programmes and for understanding natural disease dynamics) the relationship between host body temperature and ambient temperature will need to be characterised.

Several body temperature models have been developed for a number of orthopteran species. Each model uses a different method and has been parameterised using a variety of factors (such as solar radiation, wind, temperature) (see Kemp, 1986; Carruthers *et al.*, 1992; Blanford *et al.*, 1998, 2000; Samietz & Köhler, 1998; Blanford & Thomas, 1999b, 2000). It may not be possible to use some of these models since consistent records of wind speed and solar radiation in archived weather station data are not always available, therefore making it difficult to compare thermal behaviour between species and to incorporate these models in further analyses.

The aims of this chapter, therefore, are to:

1. Parameterise body temperature models for a suite of acridid species, some of which co-occur with economically important pest species that have not previously been explored, standardised to a single model.
2. Explore whether a generic model can be derived to describe a range of species (particularly those of economic importance with same preferred set point body temperature and are subject to biocontrol operations particularly for species where thermal profiles are not available).
3. Explore to what extent body temperature models might enable us to predict or prioritise species and/or habitats where biocontrol using fungal pathogens such as *M. anisopliae* var. *acridum* and *B. bassiana* is likely to be effective.

2.2 Materials and Methods

Data on thermal behaviour of 11 acridid species were derived from published records and from novel field studies. Seven of the species are considered economically important pests: *Locustana pardalina* (Walker) from South Africa, *Zonocerus variegatus* (Linnaeus) from Benin, *Oedaleus senegalensis* (Krauss) from Niger; *Nomadacris septemfasciata* (Serville) from Zambia; *Chortoicetes terminifera* (Walker) from Australia, *Dociostaurus maroccanus* (Thunberg) and *Calliptamus italicus* (Linnaeus) from Spain. The remaining species co-occur with some of the above: *Dociostaurus genei* (Ocskay) and *Euchorthippus chopardi* (Descamps) in Spain; *Acrida bicolor* (Thunberg) and *Orthochtha tunstalli* (Thunberg) in Zambia. All of the economically important species are subject to on-going biocontrol efforts

using fungal pathogens. Details of the sources of body temperature data and some key information from the respective studies are provided in Table 1.

2.2.1 Temperature measurements in the field

Novel empirical measurements of body temperatures were taken for *L. pardalina* in the Karoo, South Africa during February – April 2000, and from *D. maroccanus* and *C. italicus* in southern Spain during May – June 2000 and 2001. Data for two additional orthopteran species (*D. genei* and *E. chopardi*) that co-occurred with *D. maroccanus* and *C. italicus* were also collected during the fieldwork in Spain. Data for *N. septemfasciata* and two co-occurring species, *O. tunstalli* and *A. bicolor* were collected from Zambia during February-March 2001.

The method of measuring body temperature and environmental variables was as described in Blanford *et al*. (1998) and in subsequent publications in Table 1. In brief, body temperatures were recorded using a thermocouple connected to a hand-held, single input, fast response, digital thermometer (Omega Engineering Ltd, UK). Individual locusts and grasshoppers were captured in a small sweep net or by hand. A small hole was made in the insect's thorax using the tip of a 0.22-mm diameter hypodermic needle, and a 0.125-mm diameter copper constantan thermocouple was inserted to a depth of 2 mm. Body temperature readings were taken within 5-8s of capture when the temperature reading had stabilized. Insects held for more than 8s were discarded since body temperature might have been affected by handling. Samples were taken over a number of days throughout different hours of the day, starting before sunset and working through until 2-3 hours after sunset. Data points were therefore representative of body temperatures throughout a 24-hour period. Data were collected in the same manner for all species.

Concurrent with measuring body temperature, measurements of ambient temperatures were recorded for a range of microhabitats (soil surface and in vegetation at heights 1-cm, 15-cm and 40-cm) at the field site. These data were collected using 3-mm diameter thermisters at 1-minute intervals using two Squirrel data loggers (Grant Instruments Ltd., U.K.) that were downloaded regularly. Data from the different habitats were averaged to represent mean temperatures across the microhabitats, matched to body temperatures and used to derive species-specific body temperature models.

2.2.2 Modelling body temperatures of species

Multiple parameters, including most commonly wind speed, solar radiation and environmental temperature, can be used in developing body temperature models (see Kemp, 1986; Carruthers *et al.,* 1992; Samietz & Köhler, 1998). However, wind speed and/or solar radiation tend to improve model fit only marginally (Kemp, 1986; authors' unpublished data). Furthermore, it is rare to find consistent records of both wind speed and solar radiation in archived weather station data. Thus, in line with a number of other studies (Kemp, 1986; Blanford *et al.,* 1998; Blanford & Thomas, 2000), ambient temperature was used as the single environmental parameter in these body temperature models.

The relationship between ambient temperatures and body temperatures have been described for several acridid species using linear regressions (Anderson *et al.,* 1979). A linear relationship between ambient and body temperatures may apply for certain species and/or across certain environmental ranges (Begon, 1983; Gillis & Possai, 1983; Blanford & Thomas, 1999b). More generally, a range of non-linear functions, such as logistic regression (Kemp, 1986), polynomial regression (Carruthers *et al.,* 1992; Blanford *et al.,* 1998; Blanford & Thomas, 2000) and sigmoid functions (Samietz & Köhler, 1998) have been used to better characterise the ambient-body temperature relationship.

Polynomial regressions tend to become highly inaccurate when predicting body temperatures outside the range of temperatures used to parameterise the model, resulting in inaccurate predictions at the upper or lower end of the curve (Campbell & Madden, 1990). The logistic function is an improvement over the polynomial regression and, although good for making predictions at high temperatures (i.e. when ambient temperature exceeds body temperature) it becomes increasingly inaccurate at lower temperatures (Samietz & Köhler, 1998). The sigmoid function, as proposed by Samietz and Köhler (1998) overcomes the problems of the logistic function at lower temperatures and was, therefore, used in this study to model body temperatures during daylight hours. The sigmoid function proposed by Samietz and Köhler (1998) takes the form:

$$Tb = \frac{T_{\max} - Ta}{1 + (\frac{Ta}{T_{\inf l}})^{s}} + Ta \qquad \textbf{[Eq 1]}$$

where *Tb* is body temperature; *Ta* is mean ambient temperature at the time the body temperature was recorded; $T_{\max}$ is the asymptotic maximum body temperature; $T_{\inf l}$ is the ambient temperature at the inflection point of the sigmoid curve and *s* is the slope parameter. $T_{\max}$, $T_{\inf l}$, and *s* are parameter constants estimated, in this study, through standard non-linear procedures (Levenberg-Marquardt Estimation Method) in SPSS for Windows (v.11, 2002).

2.2.3 Data analysis

Parameters and fitted body temperature models were compared between species to evaluate preferred temperatures (*Tmax*) and the rate at which species achieve maximum temperature (*s*). Species were grouped using K-mean cluster hierarchical classification of parameter estimates (*Tmax*, *Tinfl* and *s*) in SPSS for Windows (v.11, 2002). Species with similar parameter estimates, based on clustered outputs and through visual inspection of model curves, were combined to generate generic body temperature models. Analysis of covariance (R-statistic v.1.7.0) was used to compare body temperature model predictions during the linear range of ambient temperatures (10-30°C) for different species using the groups defined with the K-mean cluster hierarchical classification.

Accuracy of generic body temperature model predictions were compared with species-specific model predictions (referred to as observed in this case) by determining the absolute mean error (*AME*) (Eq. 2) - defined as the sum of the absolute value of difference between observed and predicted body temperature for a defined period of time. The overall accuracy of the slope of the curve was determined by the root mean square error (*RMS*) (Eq. 3) (see Parton & Logan, 1981; Reicosky *et al.*, 1989; Cesaraccio *et al.*, 2001).

$$AME = \frac{\sum_{i-1}^{n} \left| (X_i^{s} - X_i^{0}) \right|}{N} \qquad \textbf{[Eq 2]}$$

$$RMS = \sqrt{\frac{\sum_{i-1}^{n}(X_i^s - X_i^0)^2}{N}} \qquad \textbf{[Eq 3]}$$

where N = total number of hourly observations, n = hourly observation, X_i^0 = predicted body temperature (by the generic model) for ith observation, and X_i^s = observed body temperature (predicted body temperature by species-specific model) for the ith observation. Small values of RMS and AME denote greater accuracy since they indicate that predicted body temperatures closely resemble observed body temperature estimates, while larger values indicate greater deviations of predictions from observed values. Generic models were accepted if they accurately predicted body temperatures across the full range of ambient temperatures (10-45°C) to within 1°C.

2.2.4 Effectiveness of fungal pathogens against species with differing thermal behaviour

The body temperature models were used to assess likely performance of biological control agents, such as *M. anisopliae* var. *acridum* or *B. bassiana* in different environments. *Beauveria bassiana* is an entomopathogenic fungus that has been used to control grasshoppers in Canada (see Inglis *et al.*, 1996). Potential efficacy of these pathogens was explored by calculating the percentage of time spent (i.e. number of hours within a 24-hour period) by each species, within their respective environments, at temperatures that are optimal and suboptimal for pathogen growth. Mean hourly temperatures for 10 days were averaged to represent a 24-hour period.

Optimal growth of *M. anisopliae* var. *acridum* (Isolate IMI330189) occurs at temperatures ranging between 20°C - 37°C; suboptimal growth occurs at temperatures less than 20°C and in excess of 37°C (see Thomas & Jenkins, 1997). For *Beauveria bassiana* (LRC 26 (Mycotech GHA isolate) shown in Fargues *et al.*, 1997) growth was shown to be optimal for temperatures ranging between 20°C - 31°C and suboptimal at temperatures below 20°C and above 31°C. Optimal and suboptimal growth limits were based on the relative growth rates for the pathogen, where optimal growth rates were defined as conditions where relative growth rates were shown to be in excess of 60% at a particular temperature.

2.3 Results

2.3.1 Ambient conditions in different environments

Environmental conditions varied between the different study sites. Day length was greatest in Spain (ca. 14 hours of day light during May-July) and remained constant throughout the study sites in Africa (ca. 12 hours). Dry subtropical desert habitats of South Africa and Australia, and the mid-latitude Mediterranean habitats of Southern Spain experienced the greatest diurnal temperature fluctuations, in excess of 30°C, with cool nights (7-16°C) and high daytime temperatures (44-50°C). The mean ambient temperatures of the dry subtropical deserts of Niger (e.g. *O. senegalensis*) were similar to those found in tropical savannah shrub land of Benin and humid sub-tropical zones of Zambia and Tanzania (e.g. *Z. variegatus* and *N. septemfasciata*), where night-time temperatures remained warm (Ta> 20°C) and daytime temperatures were warm to hot (Ta>35°C). Daily diurnal temperature fluctuations in these environments were smaller in amplitude than the semi-arid habitats, with a range of 15-20°C.

2.3.2 Preferred body temperature for individual species

Measurements of body temperature relative to ambient temperature for the 11 study species are shown in Figure 1. Minimum body temperatures ranged from 9-25°C, and maximum body temperatures ranged from 40-47°C (Figure 1). Thermal preferences (estimated from the best fit models – see below) of the different species varied by 5-10°C ranging from 34 - 41°C (Table 2a, b). A non-linear distribution of body temperature against field ambient temperatures was evident for all species, except for *Z. variegatus*, *A. bicolor* and *O. tunstalli*, which were found to be linearly distributed (see Figure 1g - i).

2.3.3 Modelling body temperature

As indicated above, most species exhibited a non-linear relationship between body temperature and ambient temperature. Parameter estimates for best-fit sigmoid models for each of these species are shown in Table 2a, while those found to have a linear relationship are shown in Table 2b. Model fit was good with $R^2 > 0.70$ (see Tables 2a and 2b).

Asymptotic maximum body temperature (*Tmax*) was highest for six of the economically important species (i.e. *L. pardalina, O. senegalensis, C. terminifera, D.*

maroccanus, *C. italicus* and *N. septemfasciata*) ranging from ~36-41°C. *Euchorthippus chopardi* and *D. genei* had *Tmax* of ~34°C (Table 2a). Ambient temperature at the inflexion point of the sigmoid curve (*Tinfl*) varied between species from ~25°C (*C. terminifera*) to ~37°C (*D. genei*) (Table 2a). *Tinfl* was lowest for four of the six economic species (*C. terminifera*, *L. pardalina*, *D. maroccanus* and *N. septemfasciata*) and highest for *D. genei* (Table 2a). The slope (*s*) also varied between the species with the steepest slopes associated with all of the economic species, except *C. italicus* (Table 2a). *Calliptamus italicus* and *D. genei* had similar slopes.

2.3.4 Model generalization

The distributions of each species in relation to the parameter estimates (*Tmax, Tinfl, s*) revealed two main clusters (Figure 2). These include similarities between four economic species (*L. pardalina, D. maroccanus, C. terminifera* and *C. italicus*) and two non-economic species (*D. genei* and *E. chopardi*). The remaining species were scattered (see Figure 2). Analysis of covariance results indicated that differences between *L. pardalina*, *D. maroccanus*, and *C. terminifera* were not significant ($F_{3,5}$ = 41.89; $p>0.05$) but were significant with *C. italicus* ($F_{4,7}$ = 350.8; $p<0.01$). Differences between *D. genei* and *E. chopardi* were also not significant ($F_{3,5}$ = 534.6; $p>0.05$).

A generic body temperature model, based on the cluster membership illustrated in Figure 2 was generated for the economic species and is summarized in Table 3. Body temperatures for *L. pardalina, D. maroccanus*, and *C. terminifera* can be described using Model A (Table 3). Model A, when compared with individual-species models was found to have an absolute mean error of < 0.4°C and root mean square error of < 0.6°C (Table 4). Regression statistics further showed that generic model predictions resembled species-specific model predictions (R^2 = 0.99, slope (*b*) ~ 1 and intercept (*a*) close to 0) (Table 4). The remaining species could not be successfully described by a generic model and were therefore not included.

One-way analysis of variance between the species with linear body temperature distributions revealed that *A. bicolor* and *O. tunstalli* were similar and could therefore be grouped but were significantly different from *Z. variegatus* ($F_{2,2182}$ = 261 $P<0.0001$, Tukey HSD $P<0.05$).

2.3.5 Implications for biological control

Figure 3a illustrates that seven (*O. tunstalli*, *A. bicolor*, *N. septemfasciata*, *Z. variegatus*, *O. senegalensis*, *D. genei*, and *E. chopardi*) of the eleven species have body temperatures that encourage optimal growth of *M. anisopliae* var. *acridum* for greater than 60% of the day to as much as 95%. The remaining species (4 of which are of economic importance) remained at sub-optimal growth conditions for 50-62% of the day.

Figure 3b illustrates that, in comparison to Figure 3a, all species spent a smaller portion of the day at temperatures optimal for *B. bassiana.* Species that were previously highly susceptible to *M. anisopliae* var. *acridum*, such as *D. genei* and *E. chopardi*, became less susceptible to *B. bassiana* under field conditions. In fact these species became as susceptible to *B. bassiana* as species with high *Tmax* values, such as *D. maroccanus*, *L. pardalina* and *C. terminifera*.

2.4 Discussion

Preferred body temperature

The aim of this study was to standardise and simplify current body temperature models for a range of new and existing species and develop a generic model that can be used to describe the thermal biology for species where there is none. Thermal behaviour was not explicitly studied but instead, models were derived to characterise the relationship between body temperature and ambient temperature in the insects' respective environments. As such, where differences are observed between species, it is not possible to determine whether these are due to innate differences in thermal biology and behaviour, to differences in habitat and environment, or a combination of both. However, from an applied perspective, one is concerned with how body temperature will influence efficacy of a biopesticide in natural field environments.

Body temperature models for the 11 species studied fell into two groups; linear and non-linear. Within the linear group (3 out of 11 species), body temperatures remained close to or slightly above ambient temperature, resembling behaviours of normothermic species. Unsurprisingly, the two species from the same habitat, *O. tunstalli* and *A. bicolor* in Zambia, had similar body temperature

distributions distinct from those of the third non-regulating species, *Z. variegatus* from Benin. The models developed for these species can be used in their respective environments, but should be used with caution if employed away from these areas. For example, Blanford *et al.*, (2000) demonstrate significantly different body temperature distributions for *Z. variegatus* populations in fallow or cassava field habitats.

Within the non-linear group (8 out of 11 species), the two non-pest species showed body temperature preferences in the range of 30-34°C. Most of the pest species, on the other hand, showed higher preferred body temperatures in the range of 38-40°C. These higher preferred temperatures are consistent with those reported for several other key pest species including *Schistocerca gregaria* (Forskål)(Chapman, 1965) and *Locusta migratoria* (Linnaeus) (Volkonsky, 1939) in Africa, and *Melanoplus sanguinipes* F., *Melanoplus packardii* (Kemp, 1986) and *Camnula pellucida* Scudder (Carruthers *et al.*, 1992) in North America. The exception to this pattern was *Nomadacris septemfasciata*, whose nymphs showed preference for a lower body temperature of 36°C. Based on earlier studies on this species (Chapman, 1959) and other species (*L. migratoria* (Chapman, 1955); *S. gregaria* (Chapman, 1965; Stower & Griffith, 1966)), it is possible that this lower preference is age related, as early instars may exhibit lower preferences than later instars and adults. In a further study, Lactin & Johnson (1995), through temperature-dependent feeding rates, suggested that 1st and 2nd instars of *M. sanguinipes* may prefer lower temperatures than 3rd and older instars, which showed a similar preferred level. However, from a control perspective insects are generally targeted aged 3rd instar or older (unless good monitoring infrastructures exist (such as in Spain, where egg pod locations are recorded by GPS each season)).

The cluster analysis and 'generic' modelling study indicated that certain species could be grouped and their Tb-Ta relationship characterized by common models. On the whole, however, the patterns were not consistent with, for example, some species sharing the same habitats falling into different clusters, whilst locust species from 3 different continents fell into the same cluster. Whether these differences are based on evolutionary physiological responses, body size (Lactin & Johnson, 1996c), preconditioning (Chapman, 1955) or seasonal differences in climate (Blanford & Thomas, 1999a) will need to be investigated further.

Species that showed similar preferred temperatures may also have other similar physiological characteristics. For example, *Melanoplus sanguinipes* in Canada (Lactin *et al.,* 1995) and *Schistocerca gregaria* in Africa (Doggett, 2001) have been shown to have similar developmental rates. Both of these species are behavioural thermoregulators with preferred body temperatures between 38-40°C (*Melanoplus sanguinipes* (Kemp, 1986; Lactin & Johnson, 1996); *Schistocerca gregaria* (Stower & Griffith, 1966; Blanford & Thomas, 1999b; Elliot *et al.*, 2002)). therefore it is likely that other species with similar thermal preferences (see above), may not only have the same rate of development but also have other intrinsic characteristics that are the same, such as fever capabilities (see Boorstein & Ewald, 1987; Blanford *et al.,* 1998; Blanford & Thomas, 1999b; Elliot *et al.,* 2002; Bundey *et al.,* 2003) and immune responses (see Gunnarsson, 1988; Hegedus & Khachatourians, 1996; Gillespie *et al.,* 2000; Ouedraogo *et al.,* 2003).

Overall, this study allows us to use species-specific and generic body temperature models to capture the effects of microclimate in which an insect resides, particularly for thermoregulators. They can be substituted into models and used to capture temperature-dependent rate processes of an insect (e.g. rate development (Lactin *et al.,* 1995), fecundity (Samietz & Köhler, 1998), etc.) that may affect population abundance and distribution (e.g. in butterflies (Bryant *et al.*, 2002)). The models may be further used to describe body temperature for species where thermal behavioural and environmental data are missing but for which basic thermal characteristics are known, such as *Schistocerca gregaria* (Stower & Griffith, 1966) and *Locusta migratoria* (Volkonsky, 1939). Both of these species are known thermoregulators with preferred body temperatures in the range of 38-40°C.

Although the results indicate that development of a single generic model to describe body temperature for a variety of locust and grasshoppers is difficult (something which would have been desirable from the practical perspective of forecasting likely effectiveness of biocontrol), the different models do, nonetheless, provide some practical insights for how well a pathogen should grow in the field and how different body temperatures can cause substantial delays to pathogen-induced mortality (see Langewald *et al.,* 1999; Arthurs & Thomas, 2000).

Practical implications for the use of biological control agents in pest management

As indicated previously, the rate of pathogen growth (see Carruthers *et al.*, 1992; Fargues *et al.*, 1997; Ouedraogo *et al.*, 1997; Thomas & Jenkins, 1997) and, hence, biopesticide performance, is mainly limited by temperature (see Blanford & Thomas, 1999b, 2000, 2001; Arthurs & Thomas, 2001a). Sub-optimal growth limits for *M. anisopliae* var. *acridum*, a widely used locust pathogen (Lomer *et al*. 2001), occur at temperatures below 20°C and in excess of 37°C with optimum growth occurring in the range of 20 to 37°C (see Thomas & Jenkins, 1997). Thus, asymptotic body temperature (*Tmax*) indicates that the majority of species in this study will severely restrict the growth of the pathogen during the day (i.e. high, medium and low *Tmax* will, to a first approximation, translate to slow, medium and fast speed of kill given they represent suitable ambient conditions). However, thermal fluctuations in the environment and the duration at *Tmax* – based on the rate at which a species heat up (*s* and *Tinfl*) are also critical factors (see Carruthers *et al.*, 1992; Inglis *et al.*, 1997). Thus, assuming all else to be equal (i.e. intrinsic susceptibility, fever capabilities, application efficiency etc.) these Tb-Ta models can provide some insight into likely pathogen performance.

Thus, in regions where large-scale aerial applications of a *Metarhizium*-based biopesticide is used to control one species, such as *D. maroccanus*, a behavioural thermoregulator with a high *Tmax*, the pathogen may have a larger impact on a non-target species such as *D. genei*, with a lower *Tmax*. Differences in species-susceptibility against not only *M. anisopliae* var. *acridum*, but also against another pathogen, *B. bassiana*, were clearly illustrated in this study by assessing the time spent by each species at temperatures that permit optimal and sub-optimal growth for the pathogen. However, a more mechanistic model that captures the effects of temperature on pathogen growth would allow for more robust analyses enabling decision-makers (such as locust control officers) to assess when and where such pathogens will be effective.

Tables and Figures

Table 1: Summary of geographic location, habitat, known thermal traits, such as preferred body temperature (*Tb*) and the source of the raw data for 11 acridid species from different regions of the world.

Family	*Species/ Geographic Location*	*Habitat*	*Age*	*Preferred Tb Temperature (°C)*	*Source*
Acrididae	***L. pardalina*** South Africa	Short-grass/ Shrub	Nymph	38-41	Blanford & Thomas, 2000 and unpublished
	N. septemfasciata Zambia	Long grass wetland	Nymph	Unknown	Raw data reanalysed from Blanford & Elliot, Imperial College unpublished
	A. bicolor Zambia	Co-occur with *N. septemfasciata*	Adult	Unknown	Raw data reanalysed from Blanford & Elliot, Imperial College unpublished
	O. tunstalli Zambia	Co-occur with *N. septemfasciata*	Adult	Unknown	Raw data reanalysed from Blanford & Elliot, Imperial College unpublished
	O. senegalensis Niger	Short-grass/ Shrub	Nymph	39	Raw data reanalyzed from Blanford *et al.*, 1998
	Z. variegatus Benin	Long-grass/ Shrub	Nymph	~37 ~35	Raw data reanalyzed from Blanford *et al.*, 2000
	C. terminifera Australia	Short-grass/ Pasture	Nymph	38-40	Raw data reanalyzed from Blanford, 2000
	D. maroccanus Spain	Short-grass/ exposed soil surface Pasture	Nymph	38-39	Blanford & Thomas, 1999b and unpublished
	C. italicus Spain	Co-occur with *D. maroccanus*	Adult	Unknown	Current study
	E. chopardi Spain	Long-grass	Adult	Unknown	Current study
	D. genei Spain	Co-occur with *D. maroccanus*	Adult	Unknown	Current study

Table 2a: Parameter estimates (with asymptotic standard errors) for the sigmoid body temperature model applied to 8 acridid species (*Locustana pardalina*, *Dociostaurus maroccanus*, *Chortoicetes terminifera*, *Oedaleus senegalensis*, *Nomadacris septemfasciata*, *Calliptamus italicus*, *Dociostaurus genei* and *Euchorthippus chopardi*). N indicates the number of body temperature measurements used in fitting the model. Further details are given in the main text.

Species	***N***	***Model parameters***			
		Tmax(±SE)	***T_infl(±SE)***	***s(±SE)***	R^2
L. pardalina	1759	41.706 (0.167)	26.751 (0.251)	-4.061 (0.158)	0.77
D. maroccanus	1199	39.648 (0.162)	27.043 (0.329)	-3.968 (0.151)	0.88
C. terminifera	822	39.735 (0.160)	25.099 (0.325)	-3.699 (0.175)	0.84
O. senegalensis	746	40.384 (0.251)	31.999 (0.483)	-6.824 (0.692)	0.78
N. septemfasciata	740	36.669 (0.217)	28.926 (0.412)	-7.335 (0.654)	0.80
C. italicus	929	38.897 (0.152)	31.155 (0.578)	-2.683 (0.192)	0.81
D. genei	264	34.622 (0.508)	37.353 (1.753)	-2.357 (0.304)	0.94
E. chopardi	274	34.968 (0.367)	33.556 (1.287)	-3.375 (0.425)	0.87

Table 2b: Linear body temperature model parameter estimates for *Zonocerus variegatus, Acrida bicolor* and *Orthochtha tunstalli.* Parameter estimates are based on a single input variable, mean ambient field temperature.

Species	***N***	***Model parameters***		
		a	***B***	R^2
Z. variegatus	1693	5.3091	0.9194	0.70
A. bicolour	77	-4.3224	1.2244	0.88
O. tunstalli	416	-1.5874	1.1391	0.82

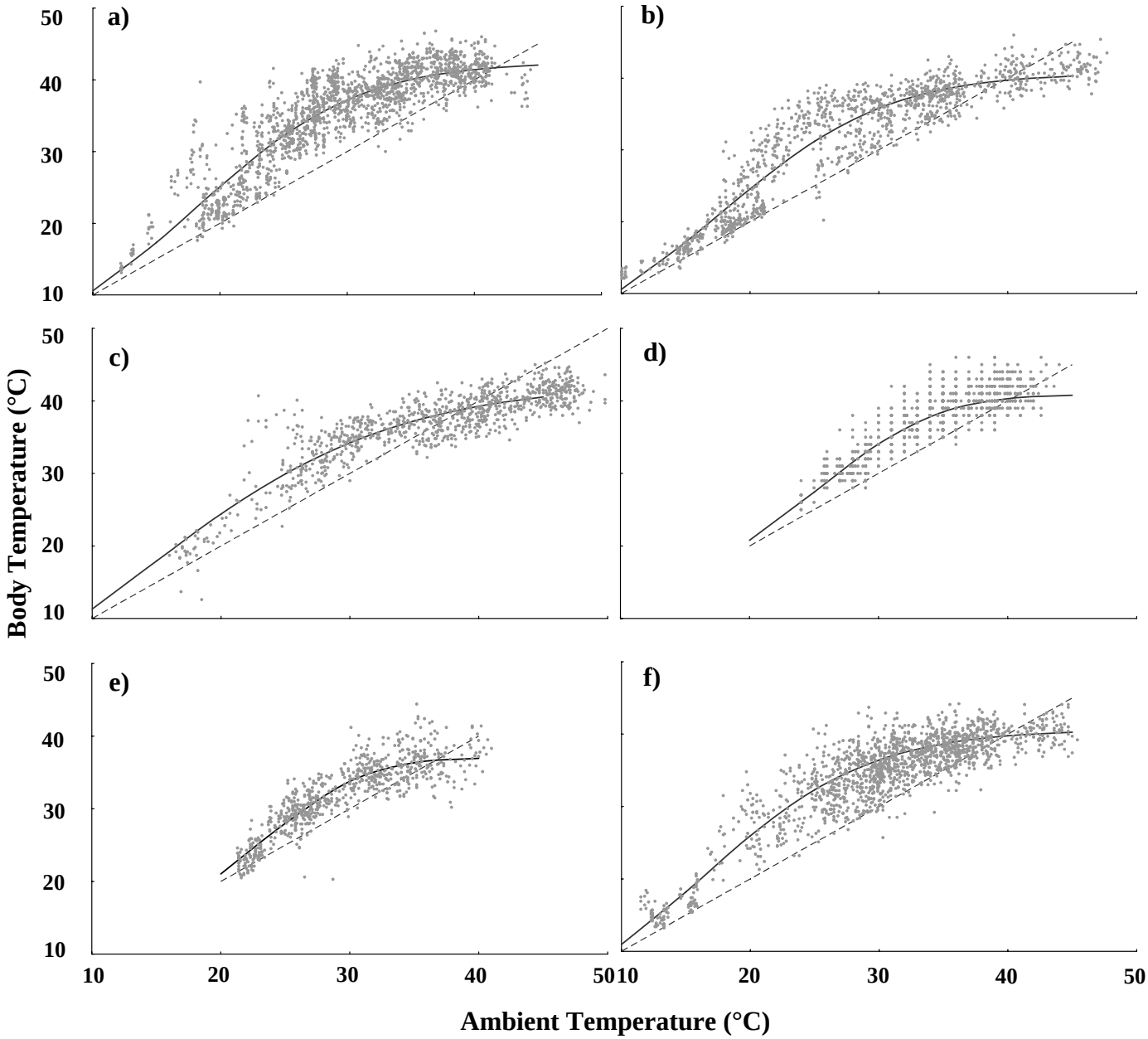
Table 3: Summary of generalised body temperature model parameter estimates for non-linear economic species with a high temperature preference, such as *Locustana pardalina, Dociostaurus maroccanus*, and *Chortoicetes terminifera*.

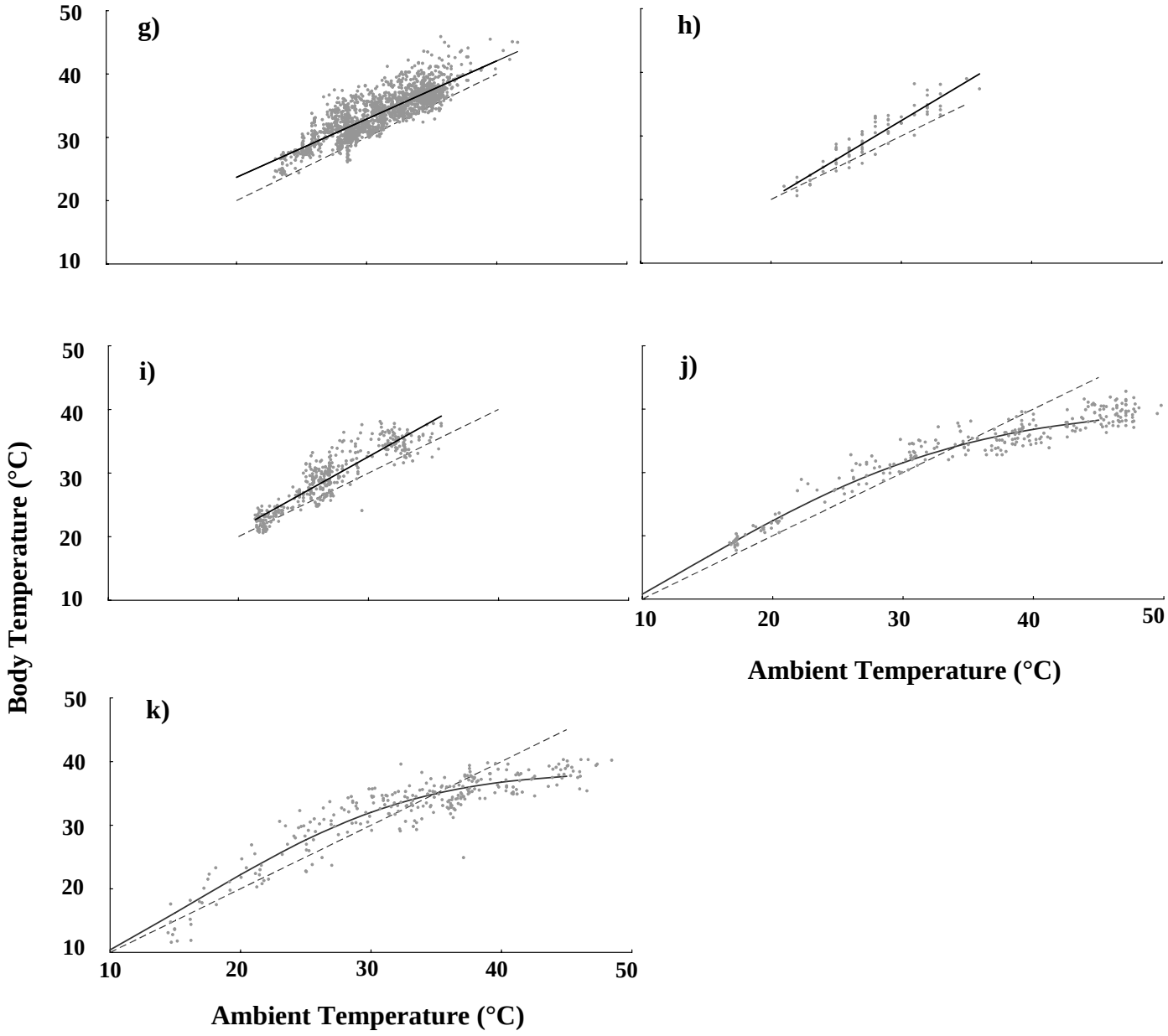
Species	***Model parameters***			
	Tmax(±SE) $(CI_{95?\%})$	***T_infl(±SE)*** $(CI_{95?\%})$	***s(±SE)*** $(CI_{95?\%})$	R^2
***Model A: High temperature preference* (38-40°C)**	40.507(0.109) (40.294-40.720)	26.185(0.185) (25.823-26.548)	-4.155(0.113) (-4.376- -3.934)	0.86

Table 4: Summary of overall accuracy of hourly predicted body temperatures using the generalised Model A against species-specific body temperature models for *Locustana pardalina* (South Africa), *Dociostaurus maroccanus* (Spain) and *Chortoicetes terminifera* (Australia). The intercept (a) and the slope (b) of the regression line, coefficient of determination (R^2), absolute mean error (AME) and root mean square error (RMS) are reported.

Species Country	***Model A***				
	a	***B***	***R^2***	***AME*±(SE) *(°C)***	***RMS (°C)***
L. pardalina *South Africa*	0.702	0.966	0.99	0.269 (0.013)	0.448
D. maroccanus *Spain*	-0.578	1.033	0.99	0.377 (0.012)	0.533
C. terminifera *Australia*	-0.385	1.015	0.99	0.232 (0.009)	0.379

Figure 1: Distribution of body temperatures (*Tb*) against ambient temperatures (*Ta*) for (a) *Locustana pardalina*, (b) *Dociostaurus maroccanus* (c) *Calliptamus italicus* (d) *Oedaleus senegalensis*, (e) *Nomadacris septemfasciata*, (f) *Chortoicetes terminifera*, (g) *Zonocerus variegates* (h) *Acrida bicolor*, (i) *Orthochtha tunstalli* (j) *Dociostaurus genei* and (k) *Euchorthippus chopardi.* The broken line (– – –) shows a null model where *Tb* = *Ta*. Best-fit body regression curves (———) for individual species were derived using the parameters in Table 2a and b.




g)
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Body Temperature (°C)
Ambient Temperature (°C)
Ambient Temperature (°C)

Figure 2 3D scatter plot illustrating the distribution of different species ((Cit) *Calliptamus italicus*; (Cte)*Chortoicetes terminifera*; (Lpa) *Locustana pardalina*; (Dma) *Dociostaurus maroccanus*; (Ose) *Oedaleus senegalensis*; (Nse) *Nomadacris septemfasciata*; (Ech) *Euchorthippus chopardi*; and (Dge) *Dociostaurus genei*) in relation to the parameter estimates (*Tmax, Tinfl* and *s*) for the individual sigmoid body temperature model (see Table 2a). Symbols are representative of cluster membership as defined using K-mean cluster analysis. Cluster 1 (●), 2(▲), 3(■), 4(✚), and 5(♦).

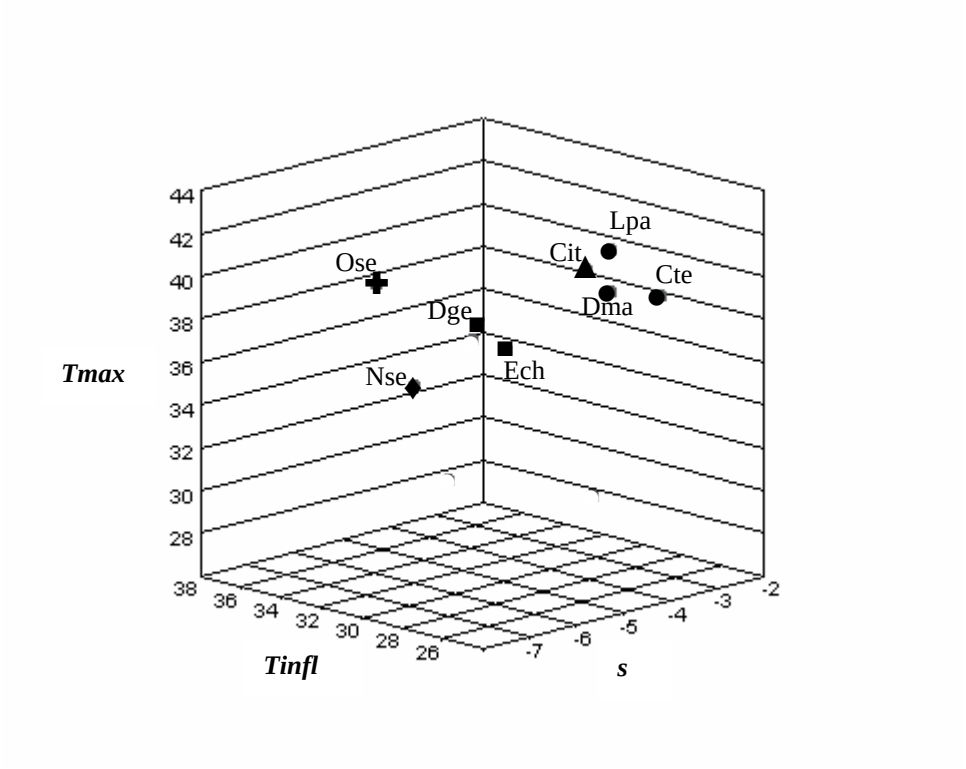
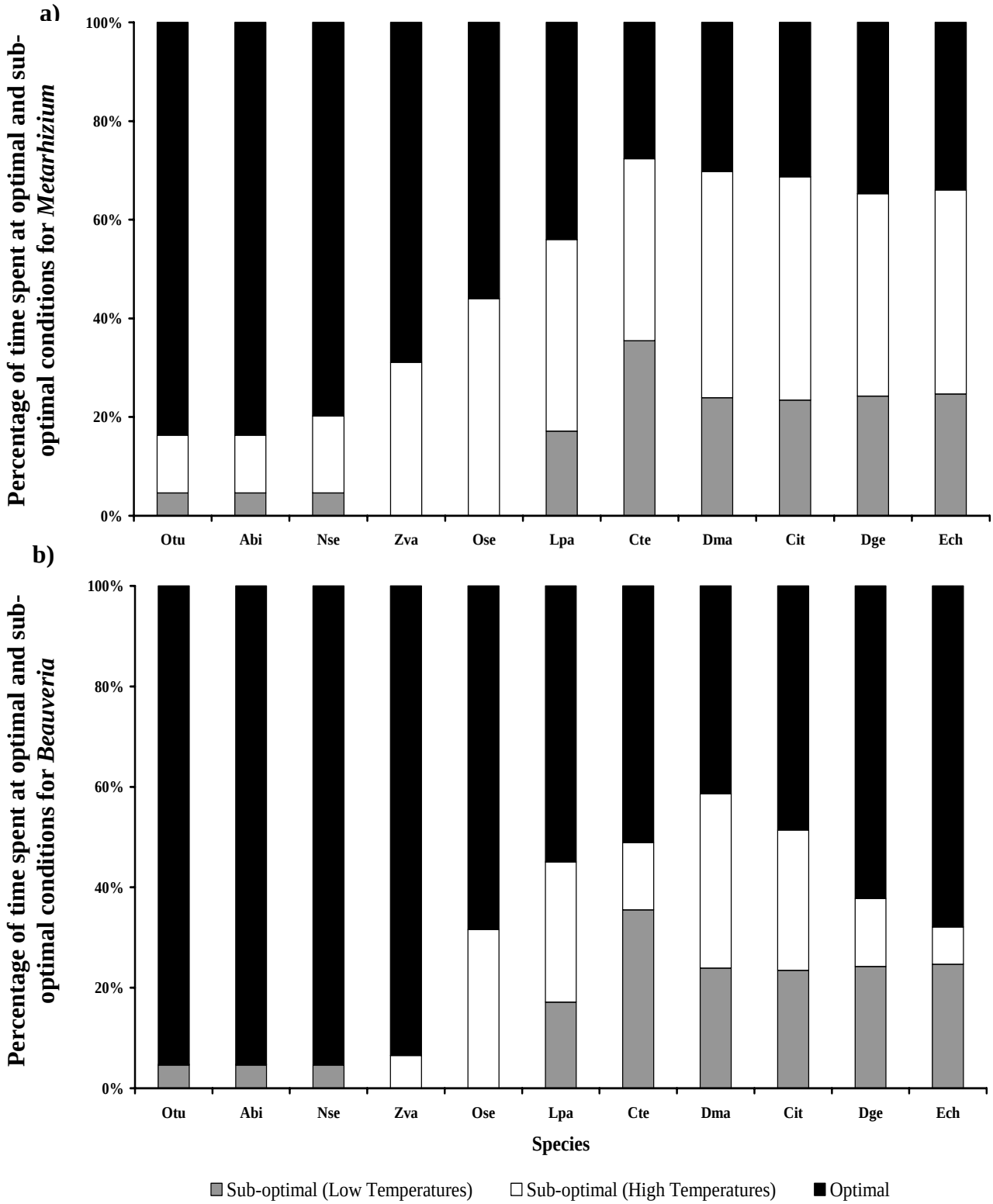


Figure 3: Percentage of time spent by an insect at temperatures that permit optimal and suboptimal growth for (a) *Metarhizium anisopliae* var. *acridum* (optimal (20°C to 37°C) and sub-optimal (20°C < *Tb* > 37°C)) and (b) *Beauveria bassiana* (optimal (20°C to 31°C) and sub-optimal (20°C < *Tb* > 31°C)) against body temperatures of different orthopteran species ((Otu) *Orthochtha tunstalli*; (Abi) *Acrida bicolor*; (Nse) *Nomadacris septemfasciata*; (Zva) *Zonocerus variegatus*; (Ose) *Oedaleus senegalensis*; (Lpa) *Locustana pardalina*; (Cte) *Chortoicetes terminifera*; (Dma) *Dociostaurus maroccanus*; (Cit) *Calliptamus italicus*; (Dge) *Dociostaurus genei*; (Ech) *Euchorthippus chopardi*).



3.0 A TEMPERATURE-DEPENDENT MODEL TO PREDICT EFFICACY OF THE PATHOGEN, *METARHIZIUM ANISOPLIAE* VAR. *ACRIDUM,* FOR BIOLOGICAL CONTROL OF LOCUSTS AND GRASSHOPPERS

ABSTRACT

1. Recent years have seen an upsurge in locust and grasshopper populations in many parts of the world. Environmentally sustainable approaches to locust and grasshopper control may be possible through the use of biopesticides based on entomopathogenic fungi. Unfortunately, performance of these biopesticides is highly variable with environmental temperature and host thermoregulatory behaviour critically determining the pattern and extent of mortality following applications. Here we present a temperature-dependent model that enables us to predict the field performance of *Metarhizium anisopliae* var. *acridum*, the key fungal pathogen used in locust biopesticides.
2. The model was constructed using mortality rate data generated across a range of temperatures in the lab and is driven by environmental temperature data linked through host body temperature models.
3. Model predictions were validated against empirical field data obtained for five species, *Locustana pardalina, Oedaleus senegalensis, Zonocerus variegatus, Nomadacris septemfasciata* and *Chortoicetes terminifera.* Mortality predictions were accurate to a 2 day error in every 10 days.
4. The model was subsequently used to investigate performance of *M. anisopliae* var. *acridum* against two additional economic species, *Dociostaurus maroccanus* and *Calliptamus italicus* in Spain. Results suggest that this pathogen would work reasonably well against *D. maroccanus* and *C. italicus.*
5. The model could provide a useful tool to develop improved application strategies to optimise the performance of the biopesticide and identify appropriate target species and environments

3.1 Introduction

In the last few years, significant locust and grasshopper problems have been reported in many parts of the world (Thomas *et al.,* 2000; Chapter 1) including, major outbreaks in Central Asia (Latchininsky & Gapparov, 2000) and parts of the US (Lorentzen, 2002). At present, control of locusts and grasshoppers is largely accomplished using chemical pesticides (Thomas & Blanford, 1998; Lomer *et al.,* 2001). However, with growing environmental and regulatory pressures, there is now substantial interest in reducing reliance on conventional pesticides and adopting more environmentally-sustainable technologies for locust and grasshopper control (Thomas & Blanford, 1998; Lomer *et al.,* 2001). To this end a number of research programmes around the world are developing biological pesticides for locust and grasshopper control using naturally occurring entomopathogens (Lomer *et al.,* 2001). These products generally have the advantage that they are specific to locusts and grasshoppers and so have negligible impact on non-target organisms (Thomas & Blanford, 1998; Thomas *et al.,* 2000; Langewald *et al.,* 2001; Lomer *et al.,* 2001). In particular, biopesticides based on the mitosporic fungus, *M. anisopliae* var. *acridum* (= *M. flavoviride* Gams and Rozsypal, Driver *et al.,* 2000), have shown considerable potential and have been tested extensively throughout Africa, Australia and parts of Europe and Latin America (Thomas *et al.,* 2000; Lomer *et al.,* 2001). Indeed, 2000 saw operational scale applications of *M. anisopliae* var. *acridum* with some 20,000 ha treated to control the migratory locust (*Locusta migratoria* (L.)) in Australia (Hunter *et al.,* 2001), with even greater areas treated in the following years (Hunter, 2001).

Unfortunately, although these new biopesticides hold considerable promise, their use is complicated by a high degree of variability in performance, a feature which characterises many biopesticides and, to some extent, biocontrol agents in general (Lisansky, 1997). Numerous studies of *M. anisopliae* var. *acridum*, as well as other fungal pathogens, have demonstrated that host thermal biology and diurnal temperature range are key factors determining efficacy (Carruthers *et al.,* 1992; Inglis *et al.,* 1996, 1997, 1999; Blanford & Thomas, 1999a, b; Arthurs & Thomas, 2000; Blanford *et al.,* 2000). For example, in the case of *M. anisopliae* var. *acridum*, under ideal temperature conditions of 25-32°C, time to death of locusts following treatment can be within 7 days (e.g. Thomas & Jenkins 1997; Bateman *et al.,* 1998; Lomer *et al.,* 2001). However, conditions in the field are not constant and temperatures may

fluctuate widely. Moreover, the majority of pest acridids are active behavioural thermoregulators (see Chappell & Whitman, 1990 for description of acridid thermal behaviour). That is, they employ a suite of behaviours to maintain a preferred body temperature, relatively independent of ambient temperatures during the day. For many species from semi-arid regions, this preferred temperature is around 38-40^{o}C (Chappell & Whitman, 1990; Carruthers *et al*., 1992; Lactin & Johnson, 1996a, b, 1998; Blanford & Thomas, 1999a, 2000). In addition, several species have also been shown to mount a behavioural fever response to infection which further enhances survival (Inglis *et al.*, 1996; Blanford *et al.*, 1998; Blanford & Thomas, 1999a, 2000; Elliot *et al.*, 2002; Ouedraogo *et al.*, 2003). Given the upper limit for growth of *M. anisopliae* var. *acridum* is in the range 35-40^{o}C (Ouedraogo *et al.*, 1997; Thomas & Jenkins, 1997), such body temperatures severely restrict pathogen development leading to a substantial delay in fungus-induced mortality (Inglis *et al*., 1996, 1997; Blanford & Thomas, 1999a,b; Arthurs & Thomas, 2000; Blanford *et al*., 2000; Ouedraogo *et al.*, 2004). The speed of kill of the biopesticide is therefore critically determined by daily temperature fluctuations (Inglis *et al.*, 1999) and the degree to which periods of pathogen growth at night, when hosts cannot thermoregulate, balance periods of minimal growth due to thermoregulation during the day. If conditions at night remain around 20-25^{o}C, as is typical of the Sahel for example (Blanford *et al.*, 1998), then pathogen growth may be rapid and mortality may still be relatively quick (Lomer *et al.*, 1997b; Langewald, *et al.*, 1999). If, however, temperatures fall below ca. 15^{o}C, pathogen growth at night will be slow exacerbating the delay still further such that mortality rate is slowed considerably (e.g. Arthurs & Thomas, 2000).

The practical significance of this variability is that standard control operations in which chemical products are sprayed and efficacy is assumed are not appropriate for *Metarhizium*-based biopesticides. Rather, successful deployment of the biopesticide requires the development of novel use strategies where expected performance parameters are defined in advance and decisions on where and when to spray adjusted accordingly (Thomas *et al*., 2000; Thomas, 2000; Lomer *et al*., 2001). In the absence of such use strategies, the conventional ‘spray and pray’ tactics will likely compound the inherent variability and critically undermine the adoption of the biopesticide technology into new integrated control practices (this argument is

supported, in part, by the current situation in South Africa where, although a locust biopesticide has been registered since 1997, adoption of the technology has been minimal (Thomas *et al.*, 2000)).

In this paper we present a model which captures the effects of environmental temperature and host thermal biology on the growth of *M. anisopliae* var. *acridum*, and enables us to predict speed of kill of the pathogen following infection. Our aim is to use this model to predict likely efficacy of biopesticide applications against different orthopteran targets in different environments, and to use this information to define effective strategies for implementing biological control of locusts and grasshoppers.

3.2 Model Overview

In this study it is assumed that a locust/grasshopper population becomes infected by *M. anisopliae* var. *acridum* following a standard biopesticide application. The application is assumed to be efficient such that all targets become infected with a lethal dose shortly after spraying. In reality, efficacy of a spray application can be quite variable but the main objective here is to understand variability in time to death following infection, and not to explore factors such as formulation, volume application rate, wind speed, temperature, operator error etc. which can affect the proportion of the population coming into contact with the biopesticide in the first place.

The primary input into the model is ambient temperature data from the field. These ambient data are then used to drive body temperature models for each key species to give the actual temperature the pathogen is exposed to once inside the infected host. The body temperatures then drive a pathogen development model which accumulates pathogen growth over a series of hourly steps until a point is reached at which either 50 or 90% mortality of the infected population is expected. This enables the effects of different environmental conditions (i.e. variation in temperature and day length over time and space) on standard measures of efficacy (i.e. LT_{50} and LT_{90}) for a range of target species to be explored. The model is validated against historical field trial data and then used in a prospective manner to explore expected pathogen performance against novel targets.

The model is written in Visual Basic, run from Excel 97. Further details of model assumptions and parameter estimation are given below.

3.2.1 Model Assumptions

As indicated above, because a massive inundation of spores following a spray application is considered, certain ecological factors such as disease transmission and age structure of the host population, which might otherwise be important in studying more natural disease dynamics is ignored. Dose is also ignored as previous studies (Thomas & Jenkins 1997; Arthurs & Thomas 2001; Blanford & Thomas 2001; Authors' unpublished data) indicate this to be of only minor importance in the overall pattern of mortality compared with temperature, at least with respect to performance in the field. Basically, if conditions are optimum for pathogen growth then even a small dose can kill locusts within 1-2 weeks. On the other hand, if conditions allow for effective host thermoregulation during the day and are then too cool for growth at night, host survival will be greatly extended no matter what the dose. Interactions with other biotic factors such as predators, which may further influence the overall extent and pattern of mortality in any given field setting (Thomas *et al.*, 1998; Arthurs & Thomas, 2001b; Arthurs *et al.*, 2003), are also excluded. Finally, a key assumption in the model is that certain aspects of the biology of the host-pathogen interaction are conserved across species. This is important as the basic data used to parameterise the effects of temperature on mortality rate are derived from lab-based studies on the desert locust, *Schistocerca gregaria* (see Arthurs & Thomas, 2001a). This assumption is valid as extensive studies on, for example, host thermal behaviour (Chapman, 1965; Stower & Griffiths, 1966; Uvarov, 1977; Kemp, 1986; Lactin & Johnson, 1996a, b, 1998; Inglis *et al.*, 1996; Blanford & Thomas, 2000), host immune response (Gunnarsson, 1988; Gillespie *et al.*, 2000; Ouedraogo *et al.* 2002, 2003, 2004), behavioural fever (Boorstein & Ewald, 1987; Blanford *et al.*, 1998; Elliot *et al.*, 2002; Bundey *et al.*, 2003), and rate of mortality (Inglis *et al.*, 1997, 1999; Blanford & Thomas, 1999a,b; Price *et al.*, 1999), show considerable similarities between many locust and grasshopper species, including those studied here.

3.2.2 Model components

(i) Thermoregulation and host body temperature.

In this study seven economically important acridid species from different geographic locations; *Locustana pardalina* (South Africa), *Oedaleus senegalensis* (Niger), *Zonocerus variegatus* (Benin), *Nomadacris septemfasciata* (Zambia), *Chortoicetes terminifera* (Australia), *Dociostaurus maroccanus* and *Calliptamus italicus* (Spain) are considered. For each of these, thermal behaviour has been characterised by measuring body temperatures in relation to ambient field temperatures, as described previously in Chapter 2. In summary, body temperature against ambient temperature showed non-linear relationships, characteristic of behavioural thermoregulators, for all species except *Z. variegatus* which is normothermic and therefore showed a linear relationship. Non-linear relationships between host body temperature and ambient temperature during the day are described using the sigmoid function, as proposed by Samietz and Köhler (1998) (refer to Section 2.2.2, Chapter 2). Parameter estimates for all seven species are presented in Tables 2a and 2b, Chapter 2. Further details of methods and data sources are given in Chapter 2.

Behavioural thermoregulation is not possible during the night. Therefore, for the hours between sunset and sunrise, body temperatures are given by:

$$Tb = Ta + 1 \qquad \textbf{[Eq 1]}$$

(ii) Rate of pathogen development

To describe the basic effects of temperature on pathogen growth (and hence mortality rate), the non-linear model proposed by Lactin *et al.* (1995) was used. This model successfully captures temperature-dependent rates for a range of processes in arthropods across the full range of environmental temperatures, and takes the form:

$$r(T) = e^{\rho T} - e^{[\rho k - (k-T)/\Delta]} + \lambda \qquad \textbf{[Eq 2]}$$

where $r(T)$ is the growth rate (per day) at temperature T, p is the Q_{10} value for a critical enzyme-catalysed biochemical reaction, k represents the thermal maximum threshold, Δ the temperature range over which thermal breakdown becomes an

overriding influence. λ allows for estimation of a developmental threshold at low temperatures by allowing the curve to intersect the abscissa at sub-optimal temperatures. *p, k,* Δ and λ are fitted parameters. Curves were fitted by iterative non-linear regression guided by partial derivatives of the dependent variable (Levenberg-Marquardt Estimation Method, SPSS v.10 for Windows, 2001) on mortality rates (1/survival time).

Mortality rates of adult desert locusts, *Schistocerca gregaria*, were determined at 5 constant temperatures (15, 20, 25, 30, 35± 1^{o}C) following infection with *M. anisopliae* var. *acridum* (isolate IMI 330189) at a dose rate of 1x10^{4} conidia/insect (Arthurs & Thomas, 2001a). No pathogen induced mortality was assumed at temperature extremes of 40 and 10°C since data from *in vitro* studies indicate that pathogen growth approaches zero at these points (Ouedraogo *et al.*, 1997; Thomas & Jenkins 1997). Survival rates expressed as 1/time (in days) (Table 1) to achieve either 50 or 90% mortality were used to parameterise Equation 2. Best fit parameters are given in Table 2, with the resulting non-linear models presented in Figure 1. Pathogen growth rate (~ 1/survival time) was constrained to ≥ 0, such that growth rate does not become negative above or below the maximum or minimum temperature for growth, respectively. Non-linear model effects were minimised by integrating constant-temperature developmental rates over fluctuating temperature regimes at hourly intervals, as proposed by Xu (1996). Thus,

$$s(rT) = \int_0^y r(T^*) = r(T)/24) \qquad \textbf{[Eq 3]}$$

where *s(rT)* is the accumulated development over the time interval [0,*y*], *r(T*)* is the hourly rate of development at a particular temperature. Accumulated fungal development *s*, is initially defined as zero and is assumed equal to 1 at the completion of the development process. Therefore, the predicted development time *(rT*)* is given by

$$s(rT^*) = 1 \qquad \textbf{[Eq 4]}$$

Accumulated development over time is approximated by

$$s(rT) = t\sum_{i=0}^{n-1} r(T^*) \qquad \textbf{[Eq 5]}$$

where $n = y / t$, t = hourly time interval and the rate of development (rT^*) is estimated from the average temperature occurring during hour (t) and accumulated over time until $s(rT) = 1$. To capture the effects of thermal behaviour, predicted body temperatures (equation 1) are substituted for ambient temperature.

Initial investigations using this model found that predictions for time to reach 50 or 90% mortality under constant temperature conditions were good, with small deviations between observed and predicted values (using the weighted least squares index (WLSI) method described by Richmond and Bacheler (1989):

$$WLSI = \sum \frac{(observed - predicted)^2}{observed} \qquad \textbf{[Eq 6]}$$

where lower WLSI values (i.e. WLSI = 0) indicate smaller deviations between observed and predicted mortality rates and higher values represent large deviations (i.e. WLSI > 5 represents an error of 7 days in 10 or 10 days in 20, etc.). For example, deviations were smallest when predicting 50 and 90% mortality at intermediate temperatures between 20-30°C (WLSI < 0.0006). However, using the model against locust survival data from fluctuating temperature experiments revealed much larger errors. Under a fixed-point fluctuation of 35/20^{o}C, for example, the model predicted a slower mortality rate than observed (WLSI = 1.8). Conversely, the model predicted a faster mortality rate than observed under a more extreme 40/20^{o}C fluctuation (WLSI > 7). Initial model predictions against empirical field data also showed the model to generally predict faster speed of kill than actually observed. Given the efforts to correct for differences between rate effects occurring at constant and fluctuating temperatures (Xu, 1996) these errors are not simply a consequence of the non-linear rate functions (Ratte, 1985). Instead, they are better attributed to biological effects of high temperatures and fluctuating conditions on the interaction

between host and pathogen, which are not captured in the constant temperature data used to parameterise the model. These are discussed below and the two correction factors derived to improve the model.

3.2.3 Model improvements

Several studies have demonstrated that exposure to high temperatures and behavioural fever appear to enhance locust/grasshopper survival beyond a simple effect of temperature reducing pathogen growth to zero (Blanford & Thomas, 1999b, 2000; Elliot *et al.,* 2002; Ouedraogo *et al.* 2003). That is, although normal preferred body temperatures around 38-40°C enhance survival by apparently restricting pathogen growth, fever temperatures some 3-4°C higher provide additional survival benefits. The mechanisms for this effect remain unclear. A recent study examining the effects of temperature fluctuations on growth of *M. anisopliae* var. *acridum in vitro*, indicated that exposures to 42^{o}C of >4 hours resulted in a disproportionately greater reduction in growth than equivalent exposures to 38^{o}C (E. Watson, unpublished PhD Thesis, University of London, 2002). This result suggests a detrimental effect of exposure to the higher temperatures on pathogen proliferation, possibly involving cellular/biochemical repair or synthesis and breakdown of heat shock proteins (Parsell & Linquist, 1993; Welch, 1993), before cell growth can resume on return to a permissive temperature. In addition, studies on locust immune responses indicate that circulation and phagocytic action of haemocytes is enhanced by thermoregulation and fever (Ouedraogo *et al.* 2003). Such enhanced immune action is likely to slow growth of the pathogen, either by reducing pathogen levels, or at least creating a delay in growth if the pathogen has, initially, to breach haemocytic encapsulations on return to permissive temperatures (Vey & Gotz, 1986).

To account for the added effects of thermoregulation and fever, two temperature-dependent delays were incorporated into the 50 and 90% mortality models. Delay 1 for the 50% mortality level states that when locust body temperatures exceed the upper limit for pathogen growth (such that pathogen growth = 0) for three or more consecutive hours, an additional three hours of no growth is assumed. For the 90% mortality model, this period of no growth is increased to four hours. For Delay 2 it is assumed that if body temperatures fall below 20^{o}C within three hours of the end of the first delay, pathogen development remains at zero until temperatures begin to

rise and are at or above 20°C. This delay applies to both 50 and 90% mortality models. The magnitude of these delays was determined by adjusting the respective models to match observed field mortality data from a trial against brown locust, *L. pardalina* (Arthurs & Thomas, 2000). As such the delays are phenomenological, but do reflect some of the likely mechanisms referred to above.

3.2.4 Model simulations

Model simulations were run using temperature data collected simultaneously with field operations against the target species in their respective habitats (i.e. the Karoo, South Africa, 1998, for *L. pardalina*; southern Niger, 1996 and 1997, for *O. senegalensis*; southern Benin, 1998 for *Z. variegatus*; La Serena, Spain, 2000 and 2001, for *D. maroccanus* and *C. italicus*; Kafue Flats, Zambia, 2001, for *N. septemfasciata*; and Hay, Australia, 2000, for *C. terminifera*). Ambient temperatures were recorded for a range of habitats (soil surface and in vegetation at various heights 1-cm, 15-cm and 40-cm) at the field sites. These were taken using 3-mm diameter thermisters at 1-minute intervals using two Squirrel data loggers (Grant Instruments Ltd., U.K.). These data were then averaged to give mean hourly temperatures at the field sites, and used to drive the model.

Model predictions were compared with actual mortality data recorded following biopesticide applications of *M. anisopliae* var. *acridum* against *L. pardalina* (Arthurs & Thomas, 2000), *O. senegalensis* (Langewald *et al.*, 1999), *Z. variegatus* (Blanford *et al.*, 2000), *N. septemfasciata* (Elliot, S.L. Pers. Comm. Imperial College) and *C. terminifera* (Hunter, D. Pers. Comm. Australian Plague Locust Commission, Australia). Accuracy of the model was assessed using the weighted least squares index (WLSI) method (see Eq 6). Full data on population mortality following biopesticide applications are not yet available for *D. maroccanus* and *C. italicus*, although preliminary data confirming susceptibility are available (e.g. see Lomer *et al*. 1999) together with appropriate environmental data and body temperature models. For these species, therefore, the model is used for prospective analyses to explore likely effectiveness of the biopesticide.

3.3 Results

3.3.1 The brown locust, Locustana pardalina*, in the Karoo, 1998*

Locustana pardalina inhabits semi-arid areas of the Karoo where temperatures can be extreme (high maximum daytime temperatures >40°C and relatively cool nights <15-20°C). During the period of February-March 1998, body temperature measurements indicated locusts were able to maintain body temperatures in the range of 38-40°C for 10 hours during the day. During the night, body temperatures fell rapidly to below 20^{o}C. Under these conditions, the model predicted a spray application of the *Metarhizium*-based biopesticide to act slowly, causing 50% mortality in 42 days and 90% mortality by day 52 (Table 3). These predictions compare very well with field mortality data following a spray application (Arthurs & Thomas, 2000) where 50% mortality was observed on day 39 (WLSI = 0.231) and 90% mortality on day 59 (WLSI = 0.237) (though it should be recognised that these field data were used to derive the delays in the model so this represents only a partial validation of the model).

3.3.2 The Senegalese grasshopper, Oedaleus senegalensis*, in southern Niger, 1996 and 1997*

Oedaleus senegalensis is the key grasshopper pest in the Sahelian region during the wet season. Measurements of ambient temperature over July-August in 1996 and 1997 revealed daily maximum temperatures frequently exceeding 35°C. Body temperature measurements showed *O. senegalensis* able to maintain body temperatures around 38^{o}C for 8 hours per day over these periods. Unlike the Karoo, however, night time ambient temperatures, and hence body temperatures, were generally above 20^{o}C. Under these conditions, the model predicted much better pathogen performance with 50% and 90% mortality of 15 and 17 days, respectively, for August 1996 and 11 and 15 days, respectively, for August 1997 (Table 3). Comparison with field trial data for the same periods and study sites (Langewald *et al.* 1999) showed these model predictions to be generally accurate with a maximum error of 4 days (Table 3).

3.3.3 The variegated grasshopper, **Zonocerus variegatus** *in southern Benin, 1998*

Zonocerus variegatus is a grasshopper pest of the humid tropical zone of west and central Africa. Measurements of ambient temperature during March-April in 1998 found daily temperatures oscillating between 22°C to 36°C. Body temperatures for *Z. variegatus* followed a linear distribution ranging between 24°C to 46°C with a mode of 36°C. Night-time temperatures in southern Benin were similar to those recorded in Niger above. Under these conditions, the model predicted that 50% and 90% mortality would be achieved in 11 and 13 days, respectively (Table 3). Comparison with field trial data for the same study period and site (Blanford *et al.*, 2000) showed model predictions to be accurate, at least at the 50% level which is all the data that are available (Table 3). However, this rate of mortality is also consistent with other field trial results in this location (Douro-Kpindo *et al.*, 1995; Lomer *et al.*, 1997a,b).

3.3.4 The Australian plague locust, **Chortoicetes terminifera***, in Australia, 2000*

Chortoicetes terminifera is a locust pest of Eastern and South-western Australia where ambient temperatures during October-November in 2000 were found to oscillate between 9°C to 41°C, similar to conditions found in the Karoo, South Africa. During this period, body temperature measurements indicated that *C. terminifera* were active thermoregulators. Body temperatures were maintained in the range of 38-40°C for 8 hours during the day. Under these conditions, the model predicted that *M. anisopliae* var. *acridum* would cause 50% mortality in 18 days and 90% mortality in 21 days at Hay, Australia (Table 3). Predictions compared favourably with field mortality data following a spray application for the same study period and site (D. Hunter, Pers. Comm., Australian Plague Locust Commission) where 50% mortality was observed on day 17 (WLSI = 0.059) and 90% mortality on day 19 (WLSI = 0.190).

3.3.5 The red locust, **Nomadacris septemfasciata** *in Zambia, 2001*

Nomadacris septemfasciata nymphs inhabit seasonally-flooded dense grassland habitats. Measurements of body temperatures indicated they were active thermoregulators and were able to maintain their body temperatures above the mean maximum temperatures by 3-5°C. However, the dense vegetation and standing water

created a thermally buffered environment that restricted heat gain during the day and heat loss at night. In general, environmental conditions appeared conducive to pathogen growth with diurnal temperatures fluctuating between 20 and 30-35°C. The pathogen performance model indicated that a spray application against red locust nymphs in February 2001 (i.e. the period of data collection) would have resulted in rapid mortality with 50% mortality by day 5 and 90% mortality by day 10 (Table 3). Predictions at the 90% mortality level were in accordance with field trial results (S. Elliot, Imperial College. Pers. Comm. and unpublished report for DFID).

3.3.6 The Moroccan locust, Dociostaurus maroccanus*, and Italian grasshopper,* Calliptamus italicus*, in La Serena, Spain*

In contrast to Zambia, field temperatures in the Spanish grassland system in La Serena tended to be highly variable, with daily maximum temperatures exceeding 40°C and minimum temperatures consistently falling below 20°C. Both *D. maroccanus* and *C. italicus* were active thermoregulators maintaining body temperatures between 38-40°C for 8-10 hours a day. Model predictions indicated that if biopesticide applications had taken place against *D. maroccanus* at the beginning of May 2000, 50 and 90% mortality would be achieved in 29 and 41 days, respectively (Table 4). For the following year, model simulations for equivalent biopesticide applications against *C. italicus* (which was much more abundant in the field in this season) indicated improved performance of the biopesticide with 50 and 90% mortality in 25 and 34 days, respectively.

3.4 Discussion

The aim of this study was to develop a model to capture the effects of environmental temperature and host-mediated behaviour on the performance (speed of kill) of a fungal-based biopesticide. There has been much interest over the years in being able to predict success and to improve effectiveness of microbial biocontrol. For example, models have been used to investigate disease dynamics (Carruthers *et al.,* 1988, 1992; Larkins *et al.,* 1995), application method and factors influencing disease prevalence (Hajek *et al.,* 1993; Barlow *et al.,* 2000; Fenton *et al.,* 2002; Scanlan *et al.,* 2002), to identify pathogens with the greatest potential (Nowierski *et al.,* 1996; Feng *et al.,* 1998), and to examine short and long term infection patterns

following spray application (Thomas *et al.,* 1995; Thomas *et al.,* 1999). However, there has been very little attention to factors determining virulence and as far as we are aware this is the first model that quantitatively captures variability in the speed of kill of a pathogen in different field environments.

The model was tested against six data sets (1 x *L. pardalina*, 2 x *O. senegalensis*, 1 x *Z. variegatus*, 1 x *C. terminifera*, 1x *N. septemfasciata*), revealing mortality predictions to be within an error of 2 days in 10 at the 50 and 90% level. There are several possible reasons for this error. These include the lack of explicit biological mechanisms characterising the host-pathogen interactions, application of the same basic model across different species and non-linear model effects. Nonetheless, the model does capture variation in mortality across a diversity of species and habitat types to a level of resolution sufficient at a practical level. That is, in terms of operational locust biocontrol and deciding whether it is appropriate to use the biopesticide or not, it is only really necessary to be able to determine whether the biopesticide will work quickly within about 10 days, more slowly in around 20 days or much worse at >30 days. Such predictions would allow locust control officers to decide whether to use the biopesticide and, for example, to optimise timing of spraying with respect to locust age. For instance, the general aim of preventive locust control is to prevent reproduction and long-range adult dispersal. To achieve this against a population of 4^{th} instar nymphs would require high levels of mortality within about 20 days. Against a population of 1^{st} and 2^{nd} instar nymphs, on the other hand, then a speed of kill of 30-40 days may be sufficient. In this context, the modelling analysis in the current study indicates that species such as *N. septemfasciata*, *Z. variegatus,* and possibly *O. senegalensis* and *C. terminifera*, might represent good targets that can be treated across the whole of the immature period, whereas species such as *L. pardalina* would need to be located and sprayed much earlier and might not be suitable for biocontrol at all.

Whilst the generality of such predictions requires further analysis, at the very least, the model could provide a useful support tool to help monitor and interpret ongoing field trials and control operations. Extending this further, using the model for prospective analysis could be very valuable given the expense of field trials, difficultly in assessing mortality against highly mobile species, and problems of introducing possibly exotic agents just for testing. That is, the model enables

predictions of likely performance based on relatively simple measures of local climatic data and body temperature. These measures can be obtained prior to any introduction or field testing, providing an initial first assessment of whether biocontrol is worth pursuing. For example, simulated outputs would suggest there is scope for control of *D. maroccanus* and *C. italicus* which, as well as being important in the Mediterranean region (Arias *et al.*, 1993; Quesada-Moraga, 1998), have been major problems in Central Asia in recent years (Latchininsky & Gapparov, 2000; The Daily Telegraph, 2000; Chapter 1).

Overall, the model presented here is a useful tool that can be used to explore and evaluate the performance of a fungal pathogen currently being used in several locust and grasshopper biocontrol programmes (see Chapter 1). To increase the utility of this model and move beyond site-specific model prediction, it will need to be linked to meteorological station data. To this end, the model has been extended and incorporated into a Geographic Information Systems (GIS) framework, which will enable area-wide applications.

Tables and Figures

Table 1: Survival times (ST) (days) and 1/ST representing 50 and 90% mortality from laboratory studies at 5 constant temperatures for *S. gregaria* infected with *Metarhizium anisopliae* var. *acridum* at a dose rate of ($1x10^4$ conidia/insect), from Arthurs & Thomas (2001).

Temperature (°C)	***50% mortality (Days)***		***90% mortality (Days)***	
	ST_{50}	**$1/ ST_{50}$**	**ST_{90}**	**$1/ ST_{90}$**
15	17.3 SE(0.56)	0.05780	20.56	0.0486
20	9 SE(0.14)	0.11111	9.7	0.1031
25	7.1 SE(0.19)	0.14085	7.8	0.1282
30	6.8 SE(0.28)	0.14706	7.6	0.1316
35	39.6* SE(3.70)	0.02525	74.6*	0.0134

* Experiment censored on day 35 with 45.5% surviving. Survival time estimated by continuing recorded mortality until number surviving = 0.

Table 2: Parameter estimates for temperature-dependent pathogen growth rate representing the rate of mortality across temperature at the 50 and 90% mortality level at a dose rate of ($1x10^4$ conidia/insect).

Mortality Level	***Pathogen growth Parameters***				
	p	***k***	Δ	***λ***	R^2
50%	0.00905	42.39639	4.30945	-1.08331	0.99
±SEM	±0.0016	±1.3705	±1.0371	±0.0280	
90%	0.00938	43.13143	4.8372	-1.09599	0.99
±SEM	±0.0024	±1.9539	±1.5783	±0.0391	

Table 3: Comparison between model predictions and mortality observed in the field for *Oedaleus senegalensis*, *Locustana pardalina*, *Zonocerus variegatus*, *Chortoicetes terminifera*, and *Nomadacris septemfasciata.* Summary of weighted least square index (WLSI) for *O. senegalensis* and *L. pardalina* predicting 50 and 90% mortality in the field.

Species	***50% Mortality (Days)***			***90% Mortality (Days)***			***Source***
	Field ST_{50}	**Model**	**WLSI**	**Field ST_{90}**	**Model**	**WLSI**	
O. senegalensis							
Niger, 1996	14	15	0.071	21	17	0.762	Langewald *et al.*, 1999
Niger, 1997	11	12	0.091	15	15	0.000	Langewald *et al.*, 1999
L. pardalina							
South Africa, 1998	39	42	0.231	59	52	0.237	Arthurs & Thomas, 2000
Z. variegatus							
Benin, 1998	10	11	0.100	-	13	-	Blanford *et al.*, 2000
C. terminifera							
Australia, 2000	17	18	0.059	19	21	0.190	Pers. Comm. David Hunter, Australian Plague Locust Commission. Agriculture, Fisheries and Forestry – Australia
N. septemfasciata							
Zambia, 2001	8	5	1.8	10	10	0	Pers Comm. Elliot, S.L, Imperial College

Table 4: Efficacy of the biopesticide against *D. maroccanus* (May-June, 2000) and *C. italicus* (May- June, 2001) during the preliminary field trials in Spain.

Species	***50% Mortality (Days)***	***90% Mortality (Days)***
D. maroccanus		
Spain, 2000	28	41
C. italicus		
Spain, 2001	25.5	34

Figure 1: Observed and predicted daily development rate of the pathogen with temperature at the 50 and 90% mortality level at a dose rate of $1x10^4$ conidia/insect. Predicted mortality was estimated using the parameters summarised in Table 3. Laboratory 1/survival time, illustrating daily proportion of pathogen growth across the range of temperatures for 50% (●) and 90% (▲) mortality are plotted against model predictions illustrating 50% (-----) and 90% (___) mortality.