

# Adult Survival, Maturation, and Reproduction of the Desert Locust *Schistocerca gregaria* Infected with the Fungus *Metarhizium anisopliae* var *acridum*

Simon Blanford and Matthew B. Thomas

Leverhulme Unit for Population Biology and Biological Control, NERC Centre for Population Biology and CABI BIOSCIENCE,  
Imperial College, Silwood Park, Ascot, Berkshire, SL5 7PY, United Kingdom

E-mail: [s.blanford@cabi.org](mailto:s.blanford@cabi.org)

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Studies were conducted with two different doses of *Metarhizium anisopliae* var *acridum* to examine the effects on survival and reproductive potential of adult *Schistocerca gregaria* under conditions that either limited thermoregulation or enabled optimal thermoregulation. Adult *S. gregaria* infected with the fungal pathogen showed either a rapid and high mortality at relatively constant temperatures or a much reduced mortality and lengthened survival time when allowed to thermoregulate. Mortality rate varied from >90% after 10 days under constant temperature conditions to 66% after 70 days under optimal thermoregulatory conditions. Effects of infection on maturation and reproduction depended on the age of the adults at the time of inoculation, the nighttime temperature regime, the fungal dose, and the length of time of the monitoring period. No difference in reproductive behaviors in treated and control insects were found in one experiment that utilized older adults and was conducted over 25 days. In a second experiment with newly fledged locusts, differences in maturation rates and total reproductive output were observed due to infection. The results from these experiments are discussed in terms of the potential of *M. anisopliae* var *acridum* to alter the balance of insect endocrine systems and the importance of the assessment of behavioral changes and their impact on microbial control agents in the long term. © 2001 Academic Press

**Key Words:** *Schistocerca gregaria*; *Metarhizium anisopliae* var *acridum*; thermoregulation; fungal pathogens; biological control; locusts; pathogenicity of; bioassay.

## INTRODUCTION

Whereas the majority of studies assessing pathogens as biocontrol agents deal with their ability to produce mortality in the target pest, a considerable number

have also assessed the impact that infection may have on host behaviors that contribute to the status of the target insect as a pest. These include studies on effects of infection on feeding (e.g., Johnson and Pavlikova, 1986), developmental time (Subrahmanyam and Ramakrishnan, 1980; Olfert and Erlandson, 1991), and fecundity (Fargues *et al.*, 1991; Zaki, 1998). However, these studies are generally conducted under environmentally constant regimes that are conducive for infection and do not consider how these behaviors and the overall impact of the pathogen might change under more realistic, variable conditions experienced in the field.

For example, in the LUBILOSA program (see Acknowledgments), which is developing a mycoinsecticide for locust and grasshopper control, previous studies have indicated that infection with the fungal entomopathogen *Metarhizium anisopliae* var *acridum* [formerly *Metarhizium flavoviride* Gams and Rozsypal, but now reclassified (Driver *et al.*, 2000)] can reduce feeding and flight ability in the desert locust *Schistocerca gregaria* Forskål (Moore *et al.*, 1992; Seyoum *et al.*, 1994). Although useful in highlighting the potential for such reductions, these studies were conducted at constant temperatures optimal for fungal development and gave the pathogen maximum opportunity not only to cause these important prelethal effects but also to induce high and rapid mortality. However, several studies have now shown speed of kill of a range of pathogens in locusts and grasshoppers to be dependent on environmental temperature and host thermal biology (Carruthers *et al.*, 1992; Inglis *et al.*, 1996, 1997, 1999; Blanford *et al.*, 1998; Blanford and Thomas, 1999a,b). Accordingly, it is likely that sub- and prelethal effects of pathogens will also be affected with potential considerable change in relative importance. Thus, there is a need to consider the impact of

*M. anisopliae* var *acridum* infections when locusts and grasshoppers are exposed to more realistic thermal regimes and where behavioral thermoregulation is made possible. The aim of this study, therefore, was to examine the effects of *M. anisopliae* var *acridum* on adult *S. gregaria* under realistic thermal regimes. Two experiments are presented in which mortality, maturation, and reproduction were assessed in adult locusts following infection.

## MATERIALS AND METHODS

### Experiment 1

This experiment was conducted at the International Institute of Tropical Agriculture in Benin, west Africa and used 10-day-old (i.e., 10 days postfledging) adult *S. gregaria* taken from a long established research colony at the institute. Four treatments were used. For the first two, 40 locusts per treatment were divided into four wood-framed cages (60 × 60 × 70 cm) with mosquito-mesh sides and roof. Each cage contained 5 male and 5 female locusts. Cages were positioned in an open-sided, covered area. Thus, they experienced ambient daylight and temperature conditions but were protected from direct sunlight, wind, and rain. Ambient temperature in these cages ranged from 28 to 33°C and no internal light or heat source was provided in the cage. One treatment was inoculated with *M. anisopliae* var *acridum*. Insects were inoculated in a standardized bioassay method similar to that described in Prior *et al.* (1995). The fungal isolate was a single conidial isolate of *M. anisopliae* var *acridum*, isolate IMI 330189. Conidial suspensions were formulated in groundnut oil and these suspensions were placed in a bath sonicator for 1 min to break up the conidial chains, and conidial counts were made with a hemocytometer. Each locust in the treatment groups received 1 μl of conidial suspension applied with a micropipette beneath the dorsal pronotal shield. Spore suspensions were adjusted to give 1 × 10<sup>3</sup> conidia per insect. Control insects were left uninoculated as previous experiments have shown no effect of the formulating oils (Blanford, 1999). These two treatments are designated as the “~30°C treatments” in future discussion.

The remaining two treatments consisted again of 40 adult *S. gregaria* of the same age and sex ratio divided between four similar cages per treatment. In these cages a 40-W light bulb was placed three-quarters of the way up the rear wall and a wire mesh climbing tube was fixed in front of the light bulb so that insects could choose a variety of perching and, therefore, thermoregulating sites. Temperatures next to and directly above the bulb reached 44–46°C while those at the bottom of the cage were 31–34°C. Nighttime temperatures fluctuated with ambient temperatures (29 ± 2°C). Light bulbs inside the cage were switched on for

9 h per day, and external lighting was switched on for 1.5 h at the beginning and end of this period. This gave a 12-h light period but locusts were limited in their ability to raise internal body temperatures to 9 h per day. One treatment was inoculated with 1 × 10<sup>3</sup> conidia of *M. anisopliae* var *acridum* in the same manner as that described above and the second treatment was left uninoculated as a control. These two treatments are designated as the “thermoregulation treatments” in future discussion.

All locusts were fed an excess of cassava (*Manihot esculenta*), the thermoregulation treatments being regularly checked to ensure that fresh food was always available as the higher temperatures caused food plants to wilt more rapidly than those in the ~30°C treatments.

Mortality was monitored every 24 h and dead insects were removed and placed in humid conditions (100% RH) at 30°C to encourage sporulation. The cumulative mortality response across the assessment period was analyzed with Kaplan–Meier survival analysis in SPSS for Windows 6.1.

Maturation rate was assessed by observation of color change in male locusts. Each locust was given a score between one (immature and mostly pink) and five (mature and fully yellow) after Norris (1954). Observations were also made on time of first copulation and time of first oviposition. Fecundity was assessed by measurement of the number of egg pods laid, eggs per pod, and successful eclosion from these pods.

### Experiment 2

This second experiment used adult *S. gregaria* that were just 3 days postfledging. Each treatment contained seven male/female pairs per cage with four cages per treatment. Cage size and layout were similar to those described above and maintained under the same light:dark regime and the same thermoregulatory regime (i.e., light bulbs were switched on for 9 h). Temperatures in the cages ranged from 33 to 46°C while the light bulbs were switched on. During the night temperatures were again left at ambient which, during the study period, averaged 20.5 ± 0.1°C, with maximum of 27.6°C and minimum of 15.8°C. Insects were hand-inoculated by the methodology described above. Three treatments were used. One treatment received 5 × 10<sup>3</sup> conidia/insect, one received 1 × 10<sup>5</sup> conidia per insect, and one was designated as an uninoculated control.

Mortality, maturation rate, and observations on mating and oviposition were monitored as above, as were egg pods laid and number of eggs per pod. No assessment was made of eclosion in this experiment.

**TABLE 1**

Average Survival Time and Significance between Treatments for Adult *Schistocerca gregaria* Experiment 1

(a) Kaplan–Meier analysis of average survival time (days ± SE) in SPSS for Windows

	Control (~30°C)	Treated (~30°C)	Control (thermoregulate)	Treated (thermoregulate)
AST (±SE)	23.45 (±0.40)	8.48 (±0.33)	24.46 (±0.19)	23.44 (±0.42)
95% Conf. Int.	22.66, 24.24	7.83, 9.13	24.08, 24.84	22.61, 24.26

(b) Log rank statistic and significance of survival times between treatments

	Control (~30°C)	Treated (~30°C)	Control (thermoregulate)
Treated (~30°C)	169.46 <i>P</i> < 0.0001		
Control (thermoregulate)	4.15 <i>P</i> = 0.042	176.55 <i>P</i> < 0.0001	
Treated (thermoregulate)	0.11 <i>P</i> = 0.074	165.56 <i>P</i> < 0.0001	5.47 <i>P</i> = 0.019

Note. Treated (~30°C) and treated (thermoregulate) were inoculated with  $1 \times 10^3$  conidia of *M. anisopliae* var *acridum* per insect.

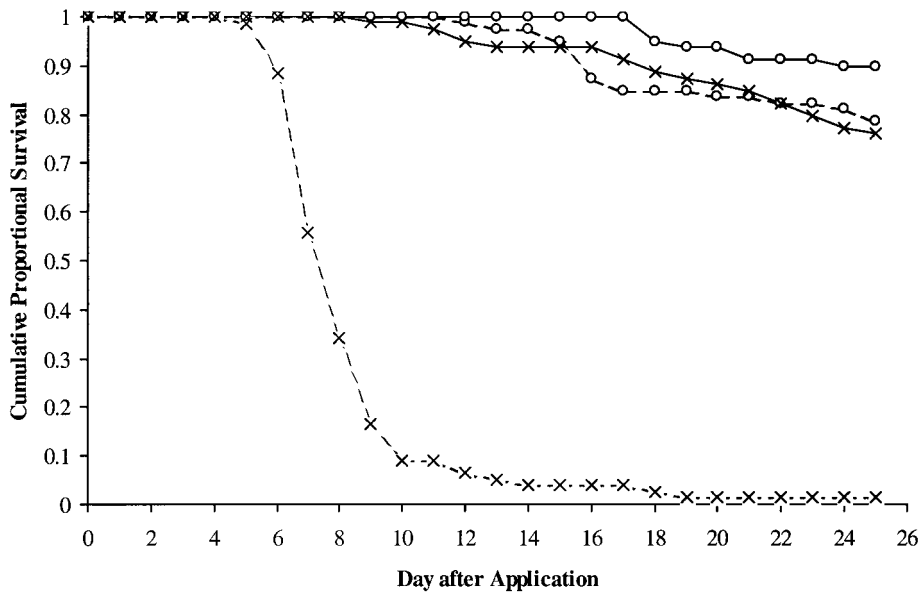
**RESULTS**

*Experiment 1*

Average survival time of control and treated locusts with their relative significance and standard errors are shown in Tables 1a and 1b. Figure 1 shows the cumulative proportional survival of the four treatments; 92.5% of cadavers from the ~30°C *M. anisopliae* var *acridum* treatment sporulated after incubation. Only 1.25% sporulated in the treated thermoregulation regime and no cadavers sporulated in either of the control treatments. Mortality in all treatments can be seen to be

significantly different from that in the treated ~30°C group which showed *M. anisopliae* var *acridum*-induced mortality commencing on day 5 and reaching 98.7% by day 19. Temperature and treatment both had a significant influence on survival time with the ~30°C control treatment and the treated thermoregulation regimes both showing significantly lower survival times than the control thermoregulation treatment. Thus, inability to thermoregulate has a direct effect on the fitness of the locusts even in the absence of the pathogen.

There was no difference in the rate at which locusts matured in the treated and control thermoregulation



**FIG. 1.** Cumulative proportional survival of adult *S. gregaria* in Experiment 1. Adults were either kept under constant conditions (30°C) without the ability to thermoregulate or kept under conditions that permitted thermoregulation (thermoregulate). Control adults were uninoculated; treated adults received  $1 \times 10^3$  *M. anisopliae* var *acridum* spores per insect. Control (30°C) (solid line and x); treated 30°C (dashed line and x); control (thermoregulate) (solid line and o); treated thermoregulate (dashed line and o) following inoculation with *Metarhizium anisopliae* var *acridum* at a range of doses.

**TABLE 2**

Average Survival Time and Significance between Treatments for Adult *Schistocerca gregaria* Experiment 2

(a) Kaplan–Meier analysis of average survival time (days $\pm$ SE) in SPSS for Windows			
	Control	$5 \times 10^3$	$1 \times 10^5$
AST ( $\pm$ SE)	64.37 ( $\pm$ 2.01)	55.89 ( $\pm$ 2.21)	50.09 ( $\pm$ 1.91)
95% Conf. Int.	60.43, 68.32	51.56, 60.22	46.35, 53.84
(b) Log rank statistic and significance of survival times between treatments			
	Control	$5 \times 10^3$	
$5 \times 10^3$	20.68		
	$P < 0.0001$		
$1 \times 10^5$	63.44	12	
	$P < 0.0001$	$P < 0.0005$	

Note. Control adults were uninoculated, *M. anisopliae* var *acridum* treatments received either  $5 \times 10^3$  or  $1 \times 10^5$  spores per insect.

treatments. All locusts started at stage two and had reached stage four/five by day 20. Locusts did not mature beyond stage two in either of the  $\sim 30^\circ\text{C}$  treatments and no eggs or reproductive behaviors were observed. All subsequent results, therefore, refer to the two thermoregulation treatments. There was no difference in time to first observed copulation (day 4 treated, day 5 controls) nor in the time of the first oviposition (day 8 for both control and treated). No difference was found for the number of pods laid during the experiment (total  $N = 80$  control and 79 treated;  $t = 0.044$ ,  $df 50$ ,  $P = 0.48$ ) nor was any difference found for the number of eggs contained within each pod (total  $N = 5541$  control and 5246 treated;  $t = 1.33$ ,  $df 157$ ,  $P = 0.09$ ). However, there was a significant difference in eclosion between the two treatments (total  $N = 4170$

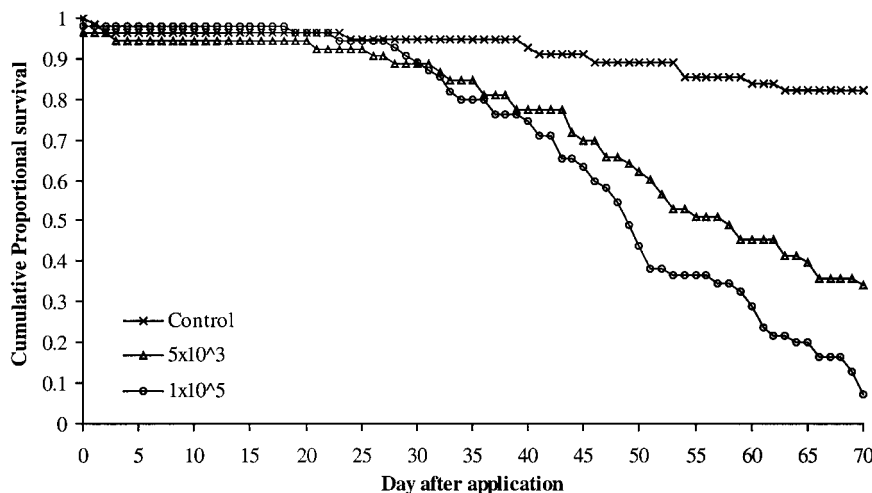
for control and 3304 for treated;  $t = 2.97$ ,  $df 157$ ,  $P < 0.002$ ).

### Experiment 2

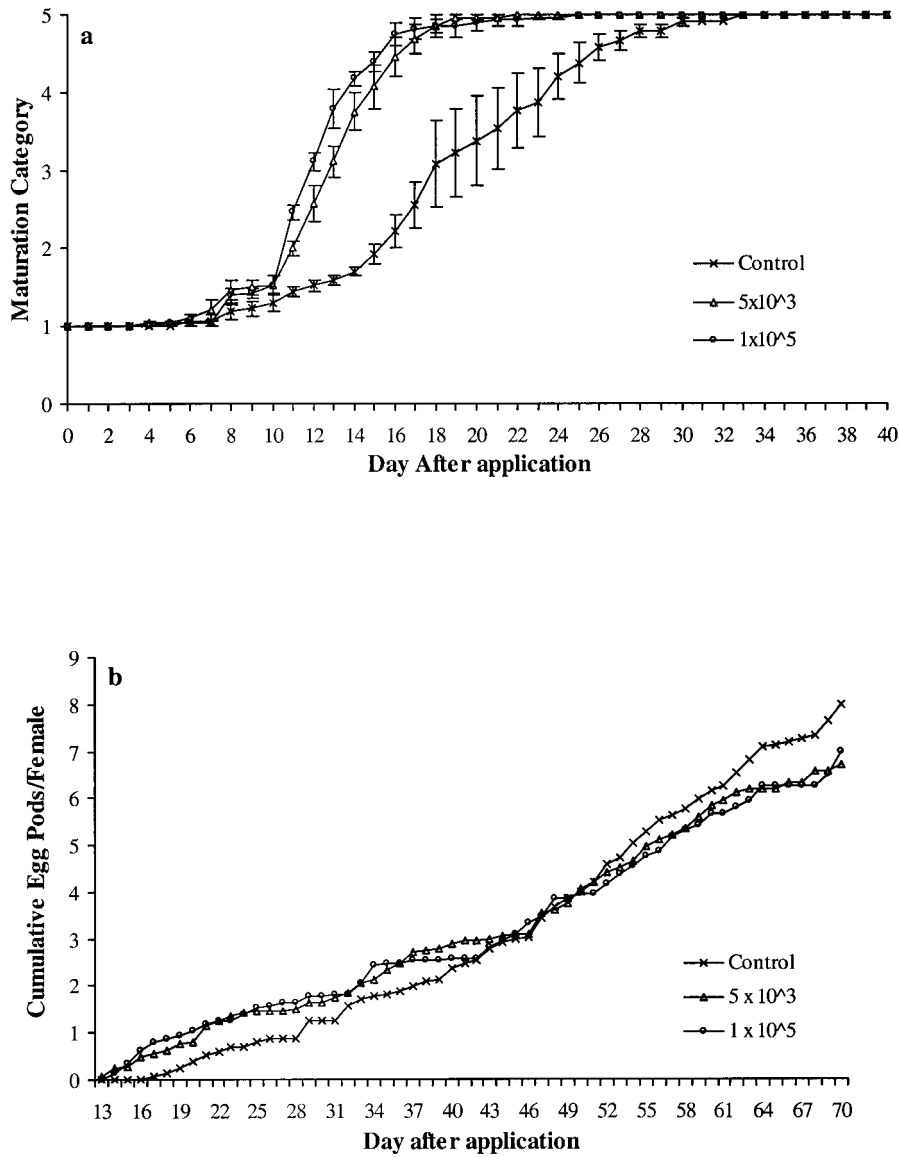
By 70 days, control mortality had reached 18.2% and mortality in the fungal treatments had reached 64.7 and 90.2% in the  $5 \times 10^3$  and  $1 \times 10^5$  treatments, respectively. *M. anisopliae* var *acridum* sporulation was observed on 18.2 and 33.3% of cadavers in the  $5 \times 10^3$  and  $1 \times 10^5$  fungal treatments, respectively. No sporulation was found for any control cadaver. Survival analysis revealed significant differences both between the *M. anisopliae* var *acridum*-treated locusts and the controls and between the two doses used in the fungal treatments (Tables 2a and 2b, Fig. 2). Analysis of average survival time over just the first 25 days (as in Experiment 1) gave no difference between any group (control 24.13 ( $\pm 0.59$ ) days;  $1 \times 10^5$  24.85 ( $\pm 0.12$ ) days;  $5 \times 10^3$  24.49 ( $\pm 0.43$ ) days;  $P > 0.05$  for all treatments).

The rate of maturation became significantly different in control and treated groups as the experiment progressed. All insects started at stage one at the beginning of the experiment. Significant differences were found after the first week with the  $5 \times 10^3$  treatment more mature than either control or the  $1 \times 10^5$  treatment ( $F_{2,81} = 3.40$ ,  $P = 0.038$ ). At day 14 both fungal treatments were significantly different from controls ( $F_{2,80} = 56.9$ ,  $P < 0.0001$ ) and this difference continued until the end of the fourth week ( $F_{2,79} = 40.14$ ,  $P < 0.0001$  and  $F_{2,79} = 6.422$ ,  $P = 0.0026$  on day 21 and day 28, respectively). All males were categorized as stage five by day 33 (Fig. 3a).

First mating was observed in both *M. anisopliae* var *acridum* treatments on day 11 and in controls on day 13. First oviposition was observed on day 13 in both *M.*



**FIG. 2.** Cumulative proportional survival of adult *S. gregaria* in Experiment 2. All adults were allowed to thermoregulate. Control adults were left uninoculated. Treated adults received either  $5 \times 10^3$  or  $1 \times 10^5$  *M. anisopliae* var *acridum* spores per insect.



**FIG. 3.** Maturation of and number of egg pods laid by *Schistocerca gregaria* in Experiment 2. (a) Rate of maturation in treated and control locusts. Maturation was ascertained by the recording of color change of male locusts. Daily values are the mean ( $\pm$ SE) of the pooled score conferred on each male locust for each treatment. (b) Cumulative number of egg pods laid per female (corrected for mortality) over the study period.

*anisopliae* var *acridum* treatments and on day 17 in the control treatment.

A significant difference was found for total number of egg pods laid in the fungal treatments and the controls ( $F_{2,210} = 27.62, P = 0.0028$ ) but no effect of dose was observed on egg pods laid in fungal treatments. When *per capita* number of pods laid was examined, no significant difference was found in any of the treatments ( $F_{2,210} = 0.0063, P = 0.59$ ). However, toward the end of the experiment, a trend toward an increased *per capita* output by control females is apparent (Fig. 3b) and it is possible that should the experiment have been run for longer this would have become significant.

Control insects laid 8756 (mean ( $\pm$ SE) per insect =  $46.8 \pm 1.01$ ) eggs. Treated insects laid 5664 ( $48.4 \pm 1.38$ ) eggs and 4851 ( $46.2 \pm 0.97$ ) eggs in the  $5 \times 10^5$  and  $1 \times 10^5$  treatments, respectively. No difference was found in any treatment for the number of eggs laid per pod ( $F_{2,406} = 0.85, P = 0.43$ ; Tukey HSD  $> 0.05$  between all treatments).

### DISCUSSION

Infected locusts died at a more rapid rate than control insects in both experiments. However, the pattern of mortality within and between experiments was



markedly different. At close to optimal temperatures for pathogen growth (Thomas and Jenkins, 1997) treated insects maintained at  $\sim 30^{\circ}\text{C}$  in experiment 1 died rapidly. Allowance for thermoregulation resulted in a reduction in overall mortality but still a significant difference from control insects allowed to thermoregulate. Interestingly, the lack of difference between control insects held at  $\sim 30^{\circ}\text{C}$  and infected insects allowed to thermoregulate highlights the fundamental importance of this behavior to the overall fitness of the insect. In contrast, survival times over the first 25 days in Experiment 2 were not different between either of the fungal treatments and controls. Thus, if the experiment had been terminated in line with Experiment 1, the actual mortality achieved across the whole study period would have been masked. Studies on fungal pathogens for control of grasshoppers and locusts have often been terminated after comparatively brief assessment periods [e.g., Fargues *et al.* (1997) monitored for 12 days in the laboratory; Delgado *et al.* (1997) monitored for 7 days in field plots; Lomer *et al.* (1997) monitored for 30 days in field plots] and as such, real control effects may have been masked.

Studies by Inglis *et al.* (1997, 1999) have demonstrated increased survival time of grasshoppers infected with a different isolate of *M. anisopliae* var *acridum* and exposed to various thermal regimes. As such they support the mortality data presented here in that with increasing temperature the rate of mortality is slower. However, in these experiments survival time is considerably longer than those of Inglis *et al.* (1997, 1999). A number of differences are apparent between these latter studies and the experiments detailed here. Doses applied in Experiment 1 and for one treatment in Experiment 2 were much lower, the length of exposure to the light bulbs was longer in these experiments, thus providing additional inhibition, and nighttime temperatures in Experiment 2 were cooler. A further difference was that in these experiments the ability to select a temperature within a gradient allowed the development of behavioral fever. This behavior has been demonstrated in *S. gregaria* (Blanford and Thomas, 1999b) and for a number of thermoregulating acridids (Blanford, 1999). Survival benefits of fever are not clear (Blanford *et al.*, 1998) but may enhance survival time [note: Boorstein and Ewald (1987) claimed survival benefits for *Melanoplus sanguinipes* infected with *Nosema acridiophagus* due to fever yet based their thermal regimes on inappropriate thermal preferred temperatures of this grasshopper (Lactin and Johnson, 1996, 1998)].

The difference in survival in treated locusts permitted to thermoregulate in Experiments 1 and 2 may be attributed to the higher nighttime temperatures in Experiment 1. It appears that though higher doses were employed in Experiment 2, the cooler nights mitigated any dose effect. This may have important con-

sequences as it highlights the fact that a considerable increase in dose will not necessarily counter balance daytime temperature effects and thermal behavior, if nighttime temperatures are themselves not conducive for pathogen growth.

The population reductions and the decrease in fecundity shown in this study may not decrease the pest status of adult *S. gregaria* in the short term [indeed, if Experiment 2 had been terminated after 25 days, treated locusts would have laid a greater number of egg pods (21, 36, and 40 for control and the two fungal treatments, respectively) and a larger number of eggs (933, 1669, and 1770 for control and the two fungal treatments, respectively)] but can be seen as highly effective in the long term. Uninfected locusts can survive for upward of 100 days and have the ability to lay eggs continually through this period (Norris, 1952). The differences found between control and treated locusts would thus be expected to increase over time. In addition, survival for infected locusts under field conditions rather than these optimal laboratory conditions is likely to be lower, and longevity reduced, in comparison to healthy locusts, thus increasing the control effects (Thomas *et al.*, 1998). This fact raises an important consideration. That is, it can be argued that assessments of fungal pathogens based on their ability to produce mortality in target insects (e.g., under bioassay conditions that favor pathogen growth—effectively the  $\sim 30^{\circ}\text{C}$  treatment in Experiment 1) present a “best case” scenario for biocontrol. As such, these experiments are useful for showing the potential suitability of the candidate pathogen for inducing mortality. However, it is clear that such survival under optimal laboratory conditions will not reflect actual survival time in the field where locusts can thermoregulate and nighttime temperatures may vary considerably from the optimum for growth. Thus, the adult experiments above can be described as “worst case” scenarios, albeit perhaps realistic scenarios at times when optimal thermoregulation is possible. Importantly, these worst case studies highlight the fact that *M. anisopliae* var *acridum*, and potentially other pathogens that may not show rapid or high mortality in the short term, may still effect considerable control of a treated population in the long term. Indeed real field environments would be expected to increase the rate of mortality and/or reduction in fecundity when there are variations in either biotic (e.g., predation) or abiotic (e.g., limitations to thermoregulation) conditions.

What emerges from this is that the screening and testing of candidate pathogens should consider both best and worst case scenarios to make an informed judgement on the suitability of agents for control under different conditions. All too often, screening and bioassays are conducted under best case scenarios, leading to false expectations and misconceptions on efficacy

and ultimately, when judged by these criteria, variable performance and even failure in the field.

Infection with *M. anisopliae* var *acridum* caused a significant change in the rate of maturation of adults in Experiment 2 but not in the initial experiment. Norris (1954) has shown that wounding *S. gregaria* can induce more rapid maturation but suggested that this was restricted to young adults and was not the case in older adults. One hypothesis to account for this is that the synthesis of juvenile hormone is affected. Maturation is controlled by this hormone, which is lacking in newly fledged adults (Nijhout, 1994). Juvenile hormone titers begin to increase after 3 to 4 days and reach a peak between 10 and 12 days after adult emergence. This period of somatic growth results in the maturation of the first oocyte (Injeyan and Tobe, 1981). At this stage visual changes can start to be seen in the resulting color change of adult males (Pener, 1967). The difference observed for the effect of *M. anisopliae* var *acridum* on maturation can, perhaps, be attributed to interference in the synthesis of juvenile hormone. If this is so, juvenile hormone titers in older adults (Experiment 1) would already have been high and further stimulus of juvenile hormone secretion may not have had an effect on the maturation process. Conversely, infection of newly fledged locusts (Experiment 2), in which juvenile hormone synthesis has either not commenced or in which titers are still low, might induce rapid production of the hormone and in turn a decrease in the time to maturation. A further complimentary hypothesis is that behavioral fever may result in an increasing rate of hormone synthesis via  $Q_{10}$  effects on the locust endocrine system. These hypotheses are currently being tested but empirical support comes from recent field work studying the effect of *M. anisopliae* var *acridum* applications on maturation and reproduction (Arthurs and Thomas, 2000).

The increased reproductive output in the first half of Experiment 2 makes sense in this context as a valuable evolutionary strategy for infected locusts. Infection is likely to increase susceptibility of locusts to factors such as predation (Thomas *et al.*, 1998) and secondary infections by other pathogens. It may also decrease the ability of the insects to migrate (Seyoum *et al.*, 1994), though this has not been studied under realistic conditions. Thus, to commence reproduction and, at least, realize part of their potential fecundity would be a useful strategy. In support of this hypothesis is the work of Adamo (1999) which has shown an increase in egg laying following bacterial infection of the cricket *Acheta domesticus*. In terms of infection with *M. anisopliae* var *acridum*, a possible cost of such an alteration in behavior is that longevity may be reduced as the adults may be, effectively, physiologically older than uninfected locusts.

Sporulation of cadavers was low in the thermoregulation treatments of both experiments in comparison to

that of the  $\sim 30^{\circ}\text{C}$  treatment of Experiment 1. Sporulation of some cadavers did occur intermittently throughout the duration of these experiments, indicating that the fungus was still active. This is supported by the fact that if direct pathogen-induced mortality (evidenced by sporulation) is removed, more treated insects still died than controls (53 and 59% mortality in comparison to 25% in the two fungal treatments and control, respectively). Infection may still have caused mortality, in cases in which cadavers did not sporulate, simply via increased stress and, possibly, as mentioned above, the effects of increased physiological age in Experiment 2. Inglis *et al.* (1999) showed the effects of incubation condition and proliferation of fungal blastospores in the insect hemocoel after infection. Though not assessed here this would be a useful technique for assessing fungal populations *in vivo* to determine fungal growth characteristics over long incubation periods. This is currently being investigated in the *S. gregaria*/*M. anisopliae* var *acridum* system (authors' unpublished data).

## CONCLUSIONS

The results presented here could be interpreted as indicating indifferent performance of *M. anisopliae* var *acridum* against adult *S. gregaria*, possibly leading to the recommendation that this pathogen should not be used for control of adult stages. However, though these studies have highlighted the fact that thermoregulation of adult locusts can prolong disease incubation considerably, control effects, in terms of ultimate mortality and fecundity, are still significant. Interpretation of the results for their long-term impact on locust populations via mortality and behavioral changes is, therefore, a more robust measure of the likely performance of this pathogen in the field.

## ACKNOWLEDGMENTS

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