

Costs and benefits of fighting infection in locusts

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ABSTRACT

Locusts and grasshoppers are truly cosmopolitan pests. In an effort to reduce the environmental side-effects of current chemical control practices, several programmes around the world are developing biopesticides, based on fungal entomopathogens, for locust and grasshopper control. Unfortunately, these biocontrol products have achieved mixed success. One of the principal reasons is that locusts are active behavioural thermoregulators, enabling them, under certain environmental conditions, to elevate their body temperatures to levels where fungal growth is suppressed. Here we develop a dynamic behavioural model to predict how locust thermoregulatory behaviour influences disease development. We use the model to explore what the overall consequences of infection might be (i.e. the net effect of disease capturing elements of both pathogen development and host defence) under different conditions in terms of locust mortality, fecundity and crop damage. We modelled two empirical fungal entomopathogens, *Metarhizium anisopliae* var. *acridum* and *Beauveria bassiana*, together with two hypothetical pathogens representing a temperature generalist and a temperature specialist. The model leads to predictions that the effects of a fungal biocontrol agent are strongly mediated by environmental temperature and host behaviour. The positive control effects are manifested through direct mortality and also sub-lethal effects on feeding and fecundity that result from modifications in behaviour associated with host defence and optimization of locust fitness. *M. anisopliae* var. *acridum* is predicted to provide the best control of locusts and the specialist fungus to provide the worst. Under hotter conditions, *B. bassiana* is predicted to provide substantially worse biocontrol than the other fungal strains. These predictions match well with empirical data. In addition, the model reveals the possibility for locusts to balance the costs of host defence through selective expression of behavioural fever in response to individual fungal diseases. We conclude that models like this one may facilitate prospective evaluation of biocontrol and advance our understanding of the role of behaviour and thermal ecology in insect–pathogen interactions.

Keywords: *Beauveria bassiana*, behavioural fever, biocontrol, dynamic state variable model, entomopathogenic fungi, locusts and grasshoppers, *Metarhizium anisopliae* var. *acridum*, thermoregulation.

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INTRODUCTION

Several studies have highlighted that temperature can play a key role in mediating the outcome of infection/parasitism in insect hosts (e.g. Hall and Bell, 1960; Schmiede, 1963; Tanada and Chang, 1968; Bronstein and Conner, 1984; Carruthers *et al.*, 1985; Samways and Grech, 1986; Boorstein and Ewald, 1987; McGuire *et al.*, 1987; Watson *et al.*, 1993; Karban, 1998). In particular, work on locusts and grasshoppers has shown that the ability of certain fungal entomopathogens to infect and kill the host depends critically on the internal body temperature and how this fluctuates with external environmental conditions. It has been shown, for example, that under certain environmental conditions, the entomophthalean pathogen, *Entomophaga grylli*, can act as the key mortality factor in populations of the variegated grasshopper, *Zonocerus variegatus* (Chapman and Page, 1979). However, even relatively subtle changes in temperature (in this case an increase in daytime maximum temperature of just 2°C at certain times of the year) allows infected hosts to recover from disease, creating an effectively immune population (Blanford and Thomas, 1999a). Similarly, studies on other locust and grasshopper species have shown that both speed of kill and overall mortality caused by mitosporic fungi such as *Beauveria bassiana* and *Metarhizium anisopliae* var. *acridum* vary greatly with changes in environmental temperature (e.g. Inglis *et al.*, 1996, 1997a,b; Blanford and Thomas, 1999a,b; Arthurs and Thomas, 2000; Blanford *et al.*, 2000). Thus, these pathogens may appear very virulent, causing extensive and rapid mortality in 5–10 days, or may appear virtually benign, with infected hosts surviving for weeks or even months. In the case of these latter pathogens, this temperature-dependent variability in virulence is of particular applied significance, since several programmes around the world are, or have been, developing these fungi as biopesticides for control of pest acridids (see Lomer *et al.*, 2001). What these programmes invariably show is that sometimes – against certain species or in certain habitats or at particular times – biopesticide applications achieve excellent control. Under other conditions, however, the same biopesticide products appear largely ineffective.

One of the reasons temperature plays such a key role in locusts and grasshoppers is that most (and certainly the vast majority that have been targeted for biocontrol using pathogens) are active behavioural thermoregulators (Chappell and Whitman, 1990). That is, they employ a suite of behaviours to maintain a preferred body temperature, relatively independent of ambient temperatures during the day. Given suitable environmental conditions, this regulatory behaviour allows many locusts (and for the sake of brevity, from here on we will refer to both locusts and grasshoppers as simply locusts) to raise body temperature to a preferred set point around 38–40°C (Chappell and Whitman, 1990; Carruthers *et al.*, 1992; Lactin and Johnson, 1996, 1998; Blanford and Thomas, 1999a, 2000). Under such conditions, growth of the biocontrol pathogens inside the host is severely restricted and there is a substantial delay in the rate of fungus-induced mortality (Inglis *et al.*, 1996, 1997a; Blanford and Thomas, 1999a,b; Arthurs and Thomas, 2000; Blanford *et al.*, 2000).

An additional dimension is that certain locusts have been shown to exhibit modified thermoregulatory behaviour and an increase in the preferred set point body temperature in response to infection (Inglis *et al.*, 1996; Blanford *et al.*, 1998; Blanford and Thomas, 1999a). This response, termed ‘behavioural fever’, has been shown to provide additional survival benefits. However, the fever response is expected to carry costs (Muchlinski, 1985; Mitchell *et al.*, 1990; Lefcort and Eiger, 1993). That is, although by raising its body

temperature a locust may reduce the growth rate of an infecting fungus and enhance its immune response allowing increased survival, the new set point is above the normal temperature optimum and will affect other processes such as feeding efficiency, growth rate and escape from predation (Boorstein and Ewald, 1987; Lactin *et al.*, 1995; Lactin and Johnson, 1995; Arthurs and Thomas, 2001a). Thus, the extent and frequency of fever is expected to be mediated by individual behavioural or physiological trade-offs. These trade-offs will depend themselves on a range of factors, such as ambient environmental temperature, temperature profile and virulence of the pathogen, and the host's age and reproductive state.

The aim of the present study was to determine how locusts respond behaviourally to balance the costs and benefits of fighting disease and how overall fitness is optimized across a season. For our model system, we adopt a biocontrol scenario where a pathogen is introduced into an adult locust population at a particular time via a spray application. However, the behaviours of the locusts and other basic biological assumptions hold also for natural disease transmission, although the pattern of infection in the population would probably differ. Our approach uses a state variable, dynamic model (Clark and Mangel, 2000) to examine how an individual's state, the temperature \times growth profile for the disease and the local climate influence locust behaviours to maximize expected fitness. Then, we simulate populations of individuals following the strategies identified by the state variable model to yield the highest fitness under a range of model scenarios with different fungal pathogens, different environments and different timing of infection. Apart from identifying how locust thermoregulation and reproduction might change in response to infection, these simulations enable us to predict the amount of damage locusts inflict on a crop and to identify which fungal strains are most effective in a particular climate.

MATERIALS AND METHODS

Dynamic programming equations

First, we outline the model in words and later translate these ideas into equations. Locusts are characterized by three states: weight w , level of infection i and age, monitored in days d and hours h . Changes in weight and infection depend on locust body temperature t . Locusts grow most rapidly when they maintain a body temperature around 38–39°C. Growth declines sharply as body temperatures exceed this. However, temperatures of 38–39°C may also permit infecting fungal populations to expand, depending on the fungal growth versus temperature profile. Since locust fitness predicted by the model depends on both body size and the level of infection at the time of reproduction, locusts face temperature trade-offs. Locusts may thermoregulate to maximize growth, but suffer reduced fitness as a result of high fungal load, or even risk dying before having time to lay eggs if the fungal load reaches a lethal level before sexual maturity. Alternatively, locusts may thermoregulate to minimize fungal load and, as a result, endure slower growth and reduced escape from predators, for example by maintaining a body temperature of 42°C. In the model, locusts balance these trade-offs by selecting the thermoregulatory behaviour to maximize expected fitness depending on their weight, fungal load and age.

Parameter and function definitions are given in Table 1. We denote the expected fitness of a locust of weight w , level of infection i , at hour h and day d as $F(w, i, h, d)$. Time (hour and day) represents locust age, which we assume parallels developmental stage (i.e. stage of

sexual maturity in the adults). Locust size (weight) varies among locusts of the same age depending on their history of infection and thermoregulatory behaviour and their initial size. At the end of the season ($h_{\max} = 24$ h and $d_{\max} = 45$ days), we assume that all locusts die, so they can expect zero future fitness, so

$$F(w, i, h_{\max}, d_{\max}) = 0 \quad (1)$$

The season length is somewhat arbitrary but is a reasonable representation of uninfected adult locust longevity (Chapman and Joern, 1990). Longer seasons have little effect on predictions about the effects of biocontrol, since fungal-induced mortality occurs many days before the end of the season.

Table 1. Variables and functions used in the model

w, i	locust weight and level of infection
h, d	hour and day of locust age
$w'(x, w, i)$	locust weight after thermoregulation to temperature x for 1 h, if the locust began the hour at weight w and fungal load i
$i'(t, i)$	level of infection after thermoregulation to temperature t for 1 h
T_a	ambient temperature
$h_{\max} = 24, d_{\max} = 45$	hours in a day and maximum locust age used in model
$m(t)$	probability a locust at temperature t is killed by a predator
$Z(i)$	metabolic cost (in terms of locust body weight) of having an infection of level i
$i_{\text{lethal}} = 10$	level of infection at which the locust dies
$i_{\max} = 11$	carrying capacity of i in the fungal growth function
$w_{\max} = 10$	maximum locust size
d_{spray}	day when biocontrol is applied
$p_{\text{perHour}}(d, d_{\text{spray}})$	probability a locust becomes infected per hour on day d , given biocontrol is applied on d_{spray}
$p_{\text{perDay}}(d, d_{\text{spray}})$	probability a locust becomes infected per day on day d , given biocontrol is applied on d_{spray}
i_0	fungal load when locust first becomes infected
k_f	constant determining how much the fungal load at the time of reproduction reduces locust fecundity
$r_{\text{Metarhizium}}(t)$	growth rate of fungus at temperature t to be used in logistic equation for fungal growth of <i>Metarhizium</i>
$r_{\text{Beauveria}}(t)$	growth rate of fungus at temperature t to be used in logistic equation for fungal growth of <i>Beauveria</i>
$r_{\text{specialist}}(t)$	growth rate of fungus at temperature t to be used in logistic equation for fungal growth of a hypothetical temperature specialist
$r_{\text{generalist}}(t)$	growth rate of fungus at temperature t to be used in logistic equation for fungal growth of a hypothetical temperature generalist
$F(w, i, h, d)$	expected fitness of a locust of weight w and fungal load i at hour h on day d
$F_{\text{feed}}(w, i, h, d)$	expected fitness if a locust thermoregulates and feeds
$F_{\text{reproduce}}(w, i, h, d)$	expected fitness if a locust reproduces
$C(w, i, h, d)$	array storing the values of thermoregulatory temperatures to maximize $F_{\text{feed}}(w, i, h, d)$
$R(w, i, h, d)$	array storing state-dependent decisions of whether or not a locust should reproduce during the specified time interval

At times before the end of the season, the model expresses fitness at hour h in terms of fitness at $h + 1$, and solves backwards through time by choosing the behaviour that maximizes expected fitness given the values of the state variables w and i , for every hour. During each hour, locusts have the option of maintaining normal thermoregulatory behaviours and continuing feeding or, if the locust is sexually mature (which we set to be at least 25 days old, which is again reasonable for a number of species; Chapman and Joern, 1990), of reproducing, after which the locust dies. Thermoregulation enables a locust to raise its body temperature up to 10°C above or down to 2°C below ambient temperature T_a (Chappel and Whitman, 1990; Blanford and Thomas, 1999a, 2000; S.N. Gardner and M.B. Thomas, unpublished data). During the night (from 18:00 until 07:00 h), locusts cannot thermoregulate; instead, their body temperature remains at 1°C above the ambient temperature (Blanford and Thomas, 1999a, 2000; S.N. Gardner and M.B. Thomas, unpublished data). They may reproduce, however. The change in fitness overnight is also calculated by backwards iteration from hour to hour. Thus, during the day, if an infected ($i > 0$) locust delays reproduction and thermoregulates to temperature t , then the expected fitness for a locust of size w , with fungal load i , at hour h and day d is

$$F_{\text{feed}}(w, i, h, d) = \max_{-2 \leq t \leq 10} \{[1 - m(T_a + t)]F(w'(T_a + t, w, i), i'(T_a + t, i), h + 1, d)\} \\ \text{for } 07:00 \leq h < 18:00 \text{ and } i > 0 \quad (2a)$$

and during the night

$$F_{\text{feed}}(w, i, h, d) = [1 - m(T_a + 1)]F(w'(T_a + 1, w, i), i'(T_a + 1, i), h + 1, d) \\ \text{for } h < 07:00 \text{ or } h \geq 18:00 \text{ and } i > 0 \quad (2b)$$

where $m(x)$ is the probability per hour that a locust with body temperature x is killed by a predator, and $w'(x, w, i)$ and $i'(x, i)$ are the new body weight and the new fungal load at the beginning of the next hour, respectively, given that the locust spends the hour at temperature x . If $w'(x, w, i) \cdot 0$ (starvation) or $i'(x, i) \cdot i_{\text{ethal}}$ (lethal infection), then $F_{\text{feed}}(w, i, h, d) = 0$.

If the locust is not infected ($i = 0$), then, in each hour of day d , it may become infected with probability $p_{\text{perHour}}(d - d_{\text{spray}})$, given that the biocontrol agent is sprayed on day d_{spray} . The probability of infection depends on the time since the biocontrol agent was sprayed because the fungus declines exponentially with a half-life of 4 days (see below). Then the locust's level of infection becomes i_0 . The locust's expected fitness during the day is

$$F_{\text{feed}}(w, 0, h, d) = \max_{-2 \leq t \leq 10} \{[1 - m(T_a + t)][(1 - p_{\text{perHour}}(d - d_{\text{spray}})) \\ F(w'(T_a + t, w, i), 0, h + 1, d) \\ + p_{\text{perHour}}(d - d_{\text{spray}}) F(w'(T_a + t, w, i), i_0, h + 1, d)]\} \\ \text{for } 07:00 \leq h < 18:00 \quad (2c)$$

and during the night

$$F_{\text{feed}}(w, 0, h, d) = [1 - m(T_a + 1)][(1 - p_{\text{perHour}}(d - d_{\text{spray}})) F(w'(T_a + 1, w, 0), 0, h + 1, d) \\ + p_{\text{perHour}}(d - d_{\text{spray}}) F(w'(T_a + 1, w, 0), i_0, h + 1, d)] \\ \text{for } h < 07:00 \text{ or } h \geq 18:00 \quad (2d)$$

The array $C(w, i, h, d) = t$ records the body temperature t that maximizes

$$F_{\text{feed}}(w, i, h, d).$$

If a locust has reached a minimum state of development for reproduction ($d = 25$ days), then it may reproduce instead of continuing to feed to increase in weight and accumulate fat body. If it reproduces, then it accrues fitness immediately, day or night, by an amount that increases with locust weight (i.e. the heavier the locust and the larger the fat body, the greater the fecundity) and decreases with fungal load according to

$$F_{\text{reproduce}}(w, i, h, d) = \max(w - k_f i, 0) \quad (3)$$

where k_f is a constant of proportionality converting a locust's fungal load into the ensuing reduction in its fecundity. For most results we used $k_f = 0.2$, but since we do not have empirical data to estimate this parameter, we also examine predictions across a range of values of k_f .

The expected fitness is the maximum of the above behavioural options:

$$F(w, i, h, d) = \max\{F_{\text{feed}}(w, i, h, d), F_{\text{reproduce}}(w, i, h, d)\} \quad (4)$$

And the behaviours are kept track of in an array recording whether a locust does better to reproduce or continue feeding:

$$R(w, i, h, d) = \begin{cases} 1 & \text{if } F_{\text{reproduce}}(w, i, h, d) \geq F_{\text{feed}}(w, i, h, d) \\ 0 & \text{if } F_{\text{reproduce}}(w, i, h, d) < F_{\text{feed}}(w, i, h, d) \end{cases} \quad (5)$$

Cycling through all integer values of w and i and backwards through time gives the solutions for expected fitness and behaviours that maximize fitness for all states and times. We used linear interpolation for non-integer values of state variables $w'(x, w, i)$ and $i'(x, i)$.

The baseline ambient temperature at every hour of the day and night was taken from field data, averaging values taken every 1–3 min over 2 days and at four levels in the canopy (Table 2). These data were collected in south-east Niger during the rainy season in 1997, from a site where there were abundant pest acridids and where *Metarhizium anisopliae* var. *acridum* was applied as a biopesticide for grasshopper control (see Blanford *et al.*, 1998, for further details). We also computed model results for the case of daytime temperatures 6°C above or below and night temperatures 3°C above or below temperatures in the baseline temperature data. In addition, we examined results from solving the dynamic model assuming that locusts could not thermoregulate, so that they remain at 1°C above ambient temperature at all times.

Changes in state variables

We assume that fungal growth inside an insect is density dependent and follows the discrete analogue of logistic growth at rate $r(t)$ at temperature t . Thus, the change in fungal load in an insect at temperature t with a level of infection i is

$$i'(t, i) = i + r(t)i(1 - i/i_{\text{max}}) \quad (6)$$

where i_{max} is the asymptotic size of the fungus (although locust death occurs before the fungus increases to this level, at the point when i first reaches $i_{\text{lethal}} < i_{\text{max}}$). Lower values of i_{lethal} would represent more virulent strains of fungus.

Table 2. Ambient hourly temperatures from empirical measurements

Hour	T_a (°C)
01:00	23.9
02:00	23.6
03:00	23.7
04:00	23.8
05:00	23.7
06:00	23.5
07:00	24.1
08:00	25.7
09:00	28.3
10:00	30.8
11:00	33.5
12:00	35.9
13:00	38.3
14:00	38.2
15:00	38.2
16:00	37.0
17:00	35.0
18:00	32.3
19:00	29.0
20:00	27.8
21:00	26.9
22:00	26.4
23:00	26.0
24:00	23.5

The locust growth rate is also assumed to follow the discrete analogue of logistic growth at a rate $h(t)$ and with a ‘carrying capacity’ of $w_{\max} + 1$. In addition, a locust pays a metabolic cost $Z(i)$ of fungal infection. Thus, feeding changes the weight of an insect according to

$$w'(t, w, i) = w + h(t)w[1 - w/(w_{\max} + 1)] - Z(i) \quad (7)$$

Weight is bounded below by 0, at which point the locust starves to death. Weight is also bounded above by a maximum body size of w_{\max} , so if $w'(x, w, i) > w_{\max}$, then we let $w'(x, w, i) = w_{\max}$. We assume that the hourly metabolic cost of infection is linearly related to the fungal load,

$$Z(i) = zi \quad (8)$$

with $z = 0.0005$. Since there are no empirical data to estimate this parameter, we examined results with a range of values. For higher values of z – for example, with $z = 0.0015$ – model predictions indicate that infected locusts ‘shrink’ and some starve to death. This is not observed in experiments. Results using $z = 0$ – that is, assuming no metabolic cost to infection – were qualitatively and quantitatively very similar to those presented, so they are not shown here.

Feeding rate of locusts

The ability of locusts to gather food and escape predation is affected by their body temperature. Empirically, it has been shown that the feeding rate rises slowly to a peak at a temperature around 38–39°C and declines rapidly thereafter (Lactin and Johnson, 1995), as can be described by the relationship

$$h(t) = \omega(e^{\rho t} - e^{[\rho T - (T-t)/\Delta]}) + \lambda \quad (9)$$

The parameters have been measured empirically to be $\rho = 0.132072$, $T = 46.0603044$, $\Delta = 7.434967$ and $\lambda = -0.463347$ (Lactin and Johnson, 1995). We calculated the scaling factor $\omega = 2.06497 \times 10^{-3}$ so that an uninfected locust growing logistically could grow from the size minimum of $w = 1$ up to w_{\max} in approximately 35 days if it experienced a favourable growth temperature of 38°C, as is observed in the laboratory for species such as the desert locust (S.N. Gardner and M.B. Thomas, personal observation).

Fungal growth rate

The growth rate of the fungal pathogen also shows a peaked dependence on temperature, although the peak occurs at a lower temperature than does the peak for the insects. We examined results calculated with four, qualitatively different forms of the relationship between fungal growth and temperature (Fig. 1). First, based on experimental measurements of the radial growth rate of *Metarhizium anisopliae* var. *acridum*, the fungal growth rate $r_{\text{Metarhizium}}(t)$ at temperature t can be described by the function

$$r_{\text{Metarhizium}}(t) = \begin{cases} \eta \frac{e^{ta}}{b + e^{(tc)^2}} & \text{for } t < 40^\circ\text{C} \\ 0 & \text{for } t \geq 40^\circ\text{C} \end{cases} \quad (10)$$

The parameters a , b and c have been determined empirically to be 5.19, 39.46 and 13.18, respectively (Thomas and Jenkins, 1997); η is a scaling factor, the calculation of which will be explained shortly. This form of the growth versus temperature relationship never becomes negative; although a locust may thermoregulate to temperatures at which the infection stops developing, the locust cannot actually reduce its current level of infection, which again is consistent with experimental observations (see Blanford and Thomas, 1999b; Arthurs and Thomas, 2001b).

We calculated the scaling factor η so that, at the optimal temperature for fungal growth (27°C), the fungal load grows logistically from the initial level i_0 at infection to the lethal load i_{lethal} in 10 days (240 h; Thomas and Jenkins, 1997):

$$i_{\text{lethal}} = \frac{i_0 i_{\max}}{i_0 + (i_{\max} - i_0) e^{-r_{\text{Metarhizium}}(27)240}} \quad (11)$$

Substituting equation (11) for $r_{\text{Metarhizium}}(27)$ and solving gives $\eta = 9.73384 \times 10^{-3}$.

A second species of fungus that has been used for locust biocontrol is *Beauveria bassiana*. Its growth rate *in vitro* peaks at 25°C at a level that is 2.5 times higher than that of *M. anisopliae* var. *acridum* (Fargues *et al.*, 1997). Like *M. anisopliae* var. *acridum*, we assume its

growth rate never becomes negative, but the minimum and maximum temperatures at which it has positive growth rates are 8°C and 35°C, respectively. We used equation (11) with $a = 2.41$, $b = 2910.26$, $c = 8.60$ and $\eta = 9.73384 \times 10^{-3}$ to describe the growth rate $r_{Beauveria}(t)$ of *Beauveria* (Fig. 1). We also modelled a scenario in which both *M. anisopliae* var. *acridum* and *B. bassiana* are applied simultaneously by using a fungal growth rate $r_{Both}(t)$ that was the maximum of $r_{Metarhizium}(t)$ and $r_{Beauveria}(t)$.

The third form of the relationship between fungal growth and temperature that we examined was a hypothetical one representing a temperature specialist (termed the ‘specialist’ fungus). At optimal temperatures, the peak growth rate is higher than that of *M. anisopliae* var. *acridum*, but the reverse is true at suboptimal temperatures (see Fig. 1):

$$r_{\text{specialist}}(t) = 0.04e^{\frac{-(t-t_{\text{opt}})^2}{2}} \tag{12}$$

The parameter t_{opt} is the temperature at which the fungus grows the fastest, which we set equal to 27°C unless otherwise specified.

The fourth, hypothetical form of the fungal growth rate that we examined was a ‘jack of all temperatures, master of none’, which we refer to as the ‘generalist’ fungus. Across a broad range of temperatures from 20°C to 36°C, fungal growth is a constant. Only at temperature extremes (below 5°C or above 45°C) does the growth rate drop linearly to zero and remain there:

$$r_{\text{generalist}}(t) = \begin{cases} \max[8 \times 10^{-4}(t - 5), 0] & \text{if } t < 20 \\ 1.2 \times 10^{-2} & \text{if } 20 \leq t \leq 36 \\ \max[1.33 \times 10^{-3}(45 - t), 0] & \text{if } t > 36 \end{cases} \tag{13}$$

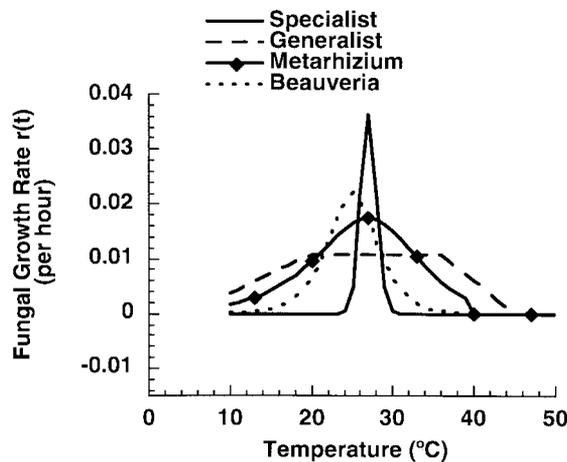


Fig. 1. Fungal growth rates $r(t)$ for *M. anisopliae* var. *acridum*, *B. bassiana*, the specialist and the generalist fungi versus temperature t .

Predation

Relatively few studies have quantified the predation experienced by locusts in the field. Some studies indicate substantial effects of predators such as birds, lizards, beetles and predatory wasps, while others indicate only minimal impact (see, for example, Chapman and Page, 1979; Farrow, 1982; Joern and Rudd, 1982; Greathead, 1992). What has been established is that body temperatures above or below optimum can alter susceptibility (Belovsky *et al.*, 1990; Forsman, 1999). Here we assume that the probability of mortality per hour due to predation is inversely related to locust performance and reaches a minimum at the temperature at which other behaviours, such as feeding rate, peak. The minimum predation rate at the temperature at which locust feeding rate is a maximum is m_0 per hour; the maximum predation rate is $m_1 + m_0$. If the maximum feeding rate is $\max_x(h(x))$, then the probability of insect mortality per hour due to predation is

$$m(t) = m_1 \left[1 - \frac{h(t)}{\max_x(h(x))} \right] + m_0 \quad (14)$$

We used $m_0 = 5 \times 10^{-4}$ and $m_1 = 5 \times 10^{-5}$, as these gave reasonable predation rates in simulated populations, within the range of predation reported in the literature.

Probability of becoming infected

Based on experimental data (see Thomas and Wood, 1997), the probability of an insect becoming infected is 30% on the day the fungal spores are sprayed (d_{spray}), and this chance declines exponentially each day with a half-life of 4 days (decay rate is $0.173 = \log(2)/4$). Thus, the probability per day of becoming infected is

$$p_{\text{perDay}}(d - d_{\text{spray}}) = 0.3e^{-0.173(d - d_{\text{spray}})} \quad (15)$$

This was converted to a per hour rate.

Forward simulations

Starting with a population of 500 locusts, the program simulated the growth, feeding rate, stochastic chance of infection and predation, fungal growth, locust thermoregulation, reproduction and mortality of each locust for every hour of days 1–45. The total crop damage was calculated as the sum of the total food consumption of all 500 locusts over the season; the number of eggs laid by the population was calculated as the sum of the reproductive fitnesses of every locust. For a given combination of parameter values, five such populations were simulated. To describe the random size variation among individual locusts at the beginning of the season, the size of each locust at $h = 1$, $d = 1$ was chosen randomly to be $w = 1, 2, 3$ or 4 with probabilities of 30%, 40%, 20% and 10%, respectively.

Dynamic programming equations

Every hour, a random number was generated and a Bernoulli trial with probability $p_{\text{perHour}}(d - d_{\text{spray}})$ determined whether or not an uninfected locust became infected. Also in each hour, locusts fed or reproduced according to the behavioural array $R(w, i, h, d)$

calculated in the solution of the dynamic programming equations. If locusts fed, then locust size and level of infection changed according to $w'(t, w, i)$ and $i'(t, i)$, depending on the thermoregulatory temperature t as specified in the array $C(w, i, h, d)$. Additionally, a Bernoulli trial with probability $m(t)$ determined whether a given locust was killed by a predator. The average size and level of infection of surviving locusts was kept track of in the variables $\bar{w}(\text{day})$ and $\bar{i}(\text{day})$, and the numbers of locusts alive, killed by predators, killed by infection and reproducing were tabulated at the end of each day.

To capture individual variation in developmental age at the time biocontrol was applied, $d_{\text{spray}}(j)$ for the j th locust was simulated stochastically for each locust to be within 5 days of d_{spray} used in solving the dynamic programming equations. Then the day of spray for the j th locust was

$$d_{\text{spray}}(j) = \text{round}([d_{\text{spray}} - 5] + \text{rnd} \cdot 10) \quad (16)$$

where rnd is a random number between 0 and 1 and $\text{round}(x)$ represents the nearest integer to x . If $d_{\text{spray}}(j) < 1$, then we let $d_{\text{spray}}(j) = 1$.

RESULTS

Locust and fungal growth

In the absence of host thermoregulation (i.e. with locust body temperature simply tracking environmental temperature), the model predicts that all fungi examined are able to kill locusts before they reproduce. When realistic thermal behaviour is added in, simulations illustrate that all except the specialist are able to kill locusts before they reproduce, provided infection occurs early enough in locust development (Fig. 2A–H). Although *M. anisopliae* var. *acridum* and *B. bassiana* do not grow very much during the day, they grow rapidly at night. The generalist grows at a more constant pace.

Since *M. anisopliae* var. *acridum* and the generalist can grow at temperatures at or even above the locusts' preferred 38–39°C, locusts infected with these fungi must initiate fevers to combat disease effectively, resulting in slower locust growth. This is not the case for *B. bassiana*, which has an upper limit for growth below the normal thermoregulatory temperatures of locusts and, as a result, locusts infected with this fungus are able to combat disease while remaining at their optimum temperatures and so grow more quickly than those infected with *M. anisopliae* var. *acridum* or the generalist. In contrast to the other fungi, the specialist fungus cannot attain lethal levels before locusts reach reproductive maturity, even if infection occurs early in locust development. Although the specialist fungus grows rapidly during certain periods at night, it fails to develop during most of the morning, day and evening, resulting in only minimal 'windows' for growth over the diurnal cycle.

Examining body temperatures in more detail, uninfected locusts and locusts infected with *B. bassiana* are predicted to thermoregulate to 38–40°C. Infection with *M. anisopliae* var. *acridum* or the generalist fungus induces a fever response increasing body temperatures to 41°C or 45°C, respectively (Fig. 3A–C). Individual variation among the simulated locusts in Fig. 3 is due to size differences among locusts (Fig. 4). Larger locusts appear to favour slightly higher temperatures. This holds for locusts regulating normally (Fig. 4 actually shows locusts infected with *B. bassiana* where there is no fever response) and, although not shown, for those exhibiting fever; larger locusts select higher fever temperatures as they are

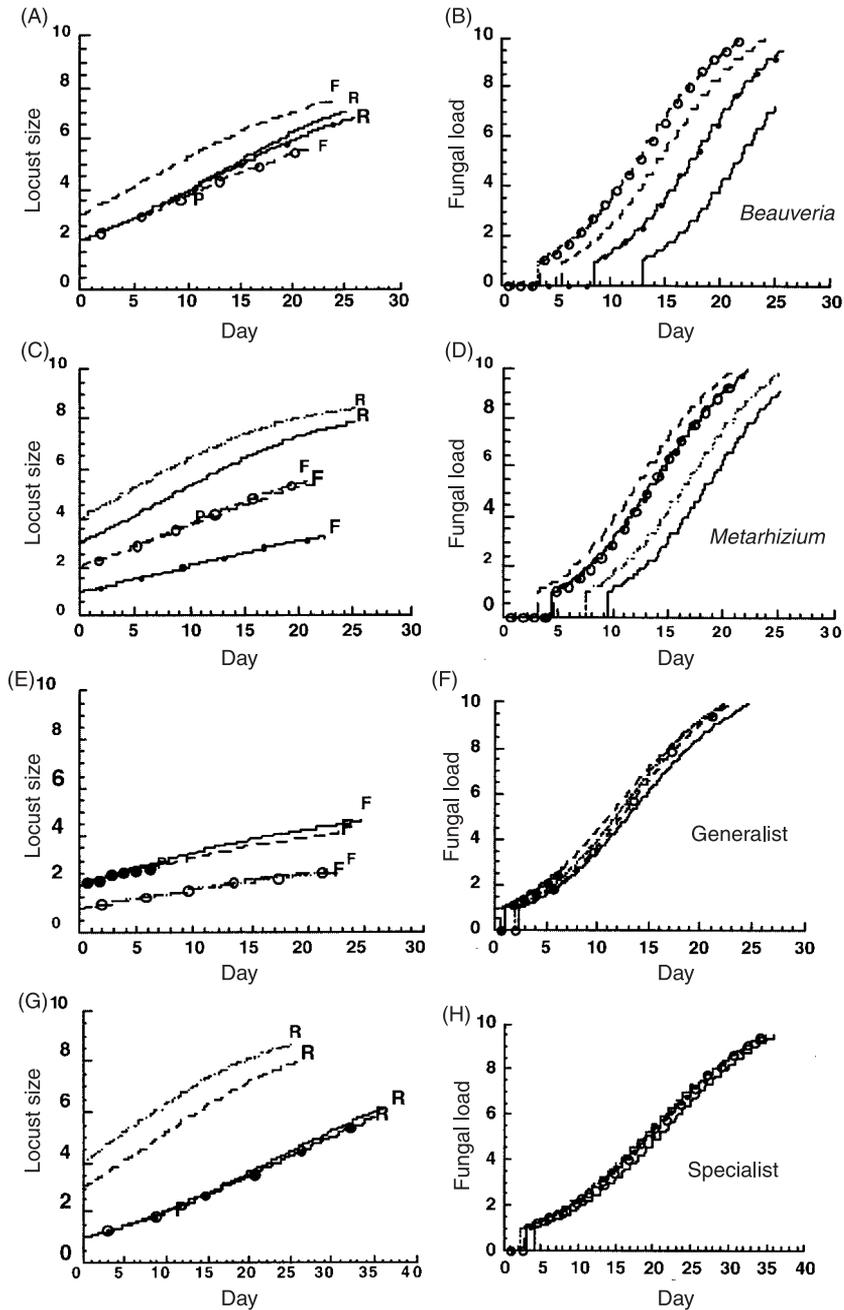


Fig. 2. Time course of locust growth (A, C, E, G) and fungal growth (B, D, F, H) of five simulated locusts exposed to *B. bassiana* (A, B), *M. anisopliae* var. *acidum* (C, D), the generalist fungus (E, F) and the specialist fungus (G, H). An F, P or R after the last plotted size of a locust indicates that the locust was killed by fungal infection, predation or reproduced, respectively. Initial locust size, whether they become infected and the time at which infection occurs, and whether they are killed by predators is determined by random simulation for each locust, as described in the 'Materials and methods'.

more able than smaller locusts to pay the cost of reduced growth rates incurred at temperatures above the normal optimum. For the fungi investigated here, the variation is not due to differences in fungal load in infected locusts. However, it is possible that for other scenarios of fungal growth, fungal load may play a role in determining the magnitude of fever. Infection with the specialist fungus does not evoke a behavioural fever response (Fig. 3D).

Causes of mortality in locust populations

In simulated populations, a combination of *M. anisopliae* var. *acridum* and *B. bassiana* is predicted to result in the highest locust mortality, followed by *M. anisopliae* var. *acridum*

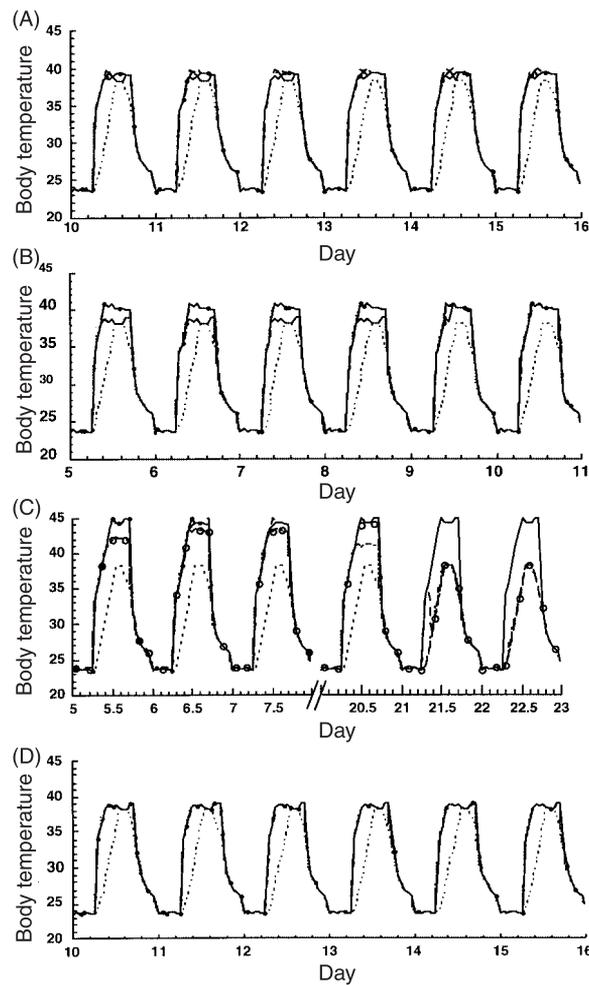


Fig. 3. Locust body temperatures and ambient temperature (dotted line) for the same five simulated locusts plotted in Fig. 2 for each type of fungus: (A) *B. bassiana*, (B) *M. anisopliae* var. *acridum*, (C) the generalist fungus and (D) the specialist fungus.

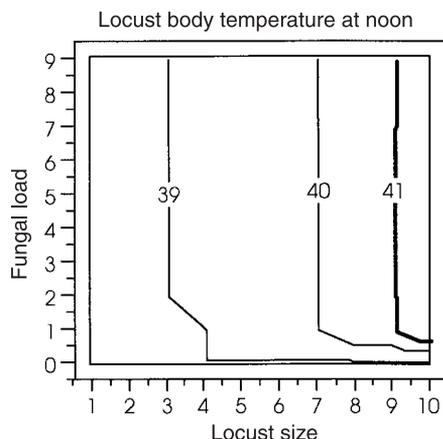


Fig. 4. Predicted locust body temperature [$C(w, i, h, d) + \text{ambient}$] in °C versus locust size and fungal load at noon on day 23 after infection with *B. bassiana*. Infection with other fungi are not plotted, but the prediction is similar that locust size matters more than fungal load in determining thermo-regulatory behaviour.

alone, then *B. bassiana*, the generalist and, finally, the specialist, which does not cause mortality directly (Fig. 5A,B). Conversely, the greatest number of individuals reproduce with the specialist, followed by the generalist, *B. bassiana*, *M. anisopliae* var. *acridum* and, finally, the combination of *M. anisopliae* var. *acridum* and *B. bassiana* (Fig. 5C). Infection with the generalist fungus is predicted to result in higher levels of reproduction than with *B. bassiana* or *M. anisopliae* var. *acridum*. With each of these three fungi, locusts are predicted to reproduce very soon after attaining reproductive maturity and earlier than they would have if they were uninfected or infected by the specialist.

Perhaps counterintuitively, infection with the specialist fungus may allow more locusts to reproduce than with no infection at all. This occurs because, in the absence of infection, locusts are predicted to delay reproduction to gain more weight and ultimately attain higher fecundity (here the simulations suggest that a few uninfected locusts may delay reproduction until age 40–45 days). However, this delay has risks in that increased longevity increases exposure to predation, so a higher proportion of locusts may be killed by natural enemies before they reproduce. The addition of density dependence in predator activity could enhance or reduce this effect, depending on the nature of the density-dependent response (although with the type of generalist predation we have assumed here, strong density-dependent effects are considered unlikely). On the positive side, from a control perspective, although locust populations treated with the specialist may not die from disease and may have higher numbers reproducing, because they reproduce earlier and at smaller sizes, damage and fecundity are reduced by 10% and 30%, respectively, relative to no disease (Fig. 6).

The generalist and *M. anisopliae* var. *acridum* are predicted to reduce damage the most, by 35% relative to no disease, while *M. anisopliae* var. *acridum* and *B. bassiana* are predicted to reduce locust reproduction the most, by approximately 80%. The reason the generalist is more effective than *B. bassiana* at reducing damage – and, conversely, why *B. bassiana* decreases reproduction more than the generalist – is that the generalist forces locusts to

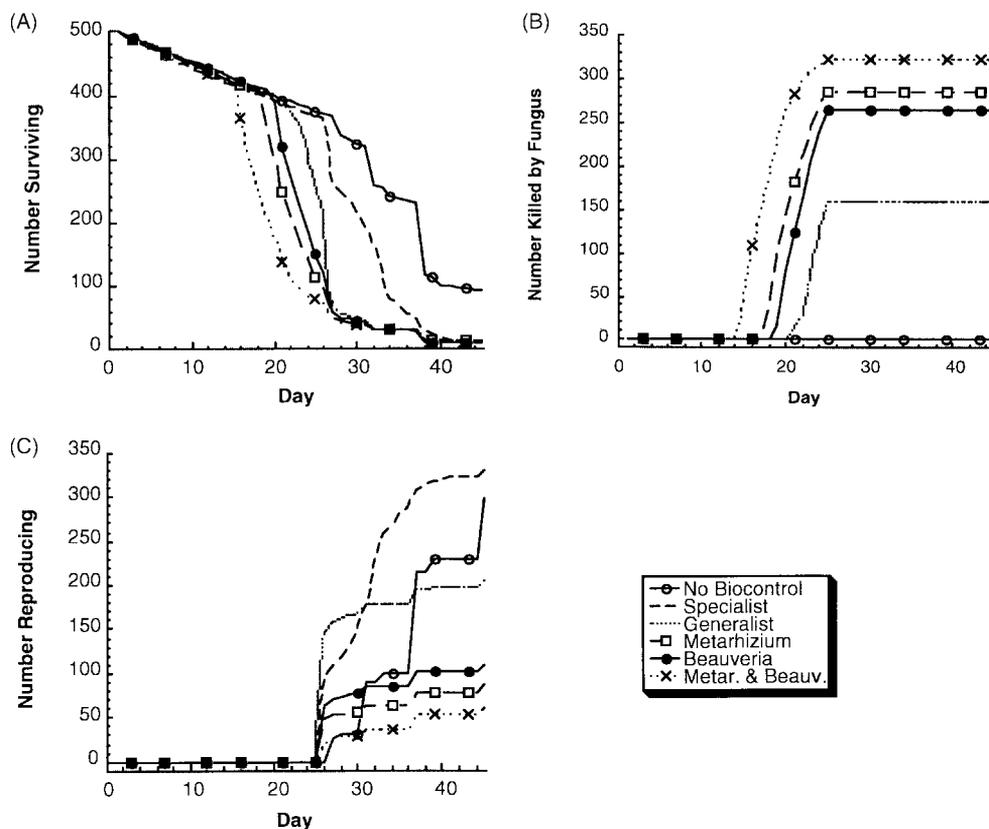


Fig. 5. (A) The number of locusts alive versus time, (B) the number killed by infection and (C) the number that reproduce in simulated populations exposed to different fungal strains or to no biocontrol. Error bars are approximately ± 10 or less and are omitted for clarity.

thermoregulate to such high temperatures that, although more survive to reproduce, they are very inefficient consumers.

Other parameters

Building on the results above, we find that as well as the biological characteristics of fungus used, damage and total fecundity depend on the time (in terms of locust development) that fungal spores are sprayed (Fig. 7). In general, the earlier infection occurs, the greater the effect in terms of damage, fecundity and mortality.

We also examined results for different values of the temperature optimum for a specialist fungus by varying the optimal temperature, t_{opt} , in equation (13). Crop damage and locust fecundity reached a nadir when $t_{\text{opt}} = 24^{\circ}\text{C}$ (Fig. 8). Although the specialist cannot grow during the day with this value of t_{opt} , this profile does allow it to expand rapidly at night. Nevertheless, reductions in damage and fecundity by the specialist with $t_{\text{opt}} = 24^{\circ}\text{C}$ do not match those achieved by the generalist, *M. anisopliae* var. *acidum* or *B. bassiana*.

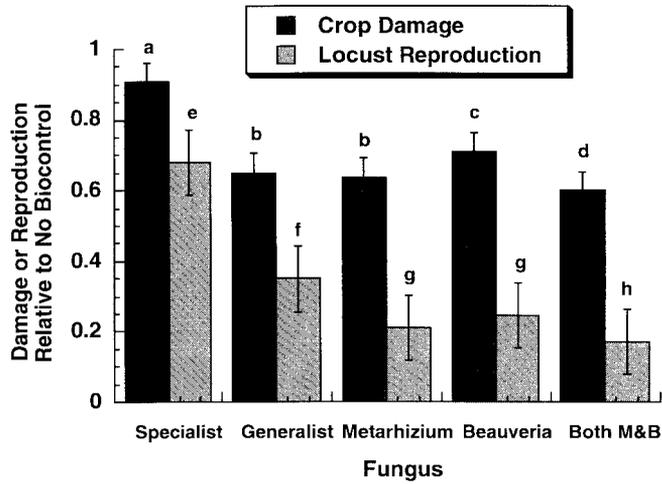


Fig. 6. Crop damage and locust fecundity relative to that without biocontrol for each fungus. Different letters indicate that means are significantly different ($\alpha = 0.05$) by the Tukey-Kramer HSD method calculated using JMP Statistical Software.

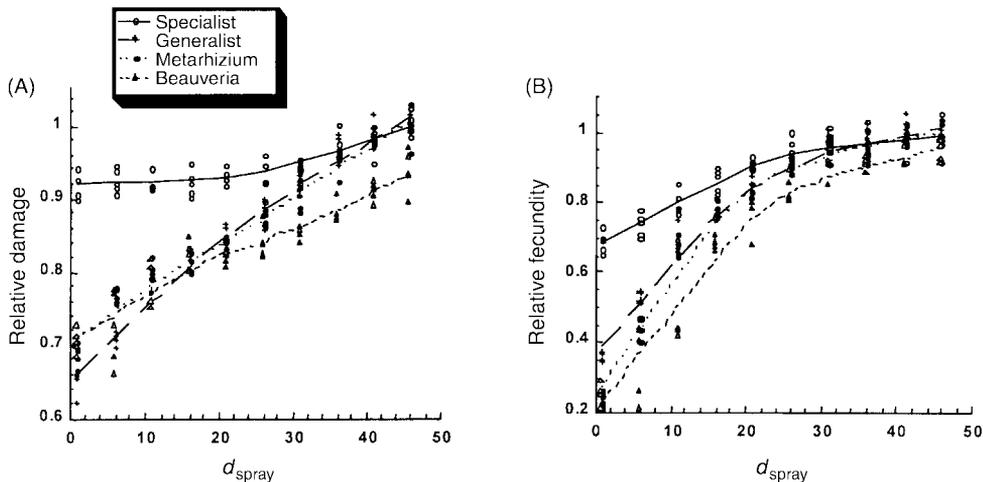


Fig. 7. Simulation results showing (A) levels of crop damage and (B) summed fecundity of all locusts in a population versus the day d_{spray} that biocontrol is applied, relative to the mean of five simulated populations in which no biocontrol is sprayed. Spraying early in the season is predicted to enable fungi to reach lethal levels before locusts attain reproductive maturity. Each point represents one simulated population.

Varying the parameter k_i , the metabolic cost of infection per unit of fungal load (equation 3), has no effect on the predicted amount of damage (Fig. 9A). Higher metabolic costs of infection do lead to decreases in locust fecundity, although these declines are small for all but the specialist and generalist fungi (Fig. 9B).

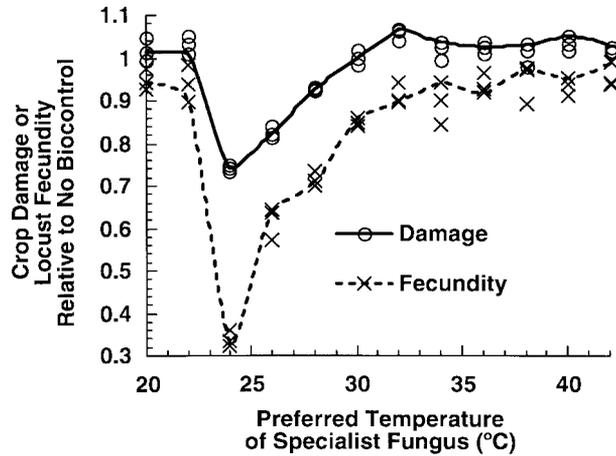


Fig. 8. Crop damage or locust fecundity versus the temperature t_{opt} at which the growth rate of the specialist fungus peaks.

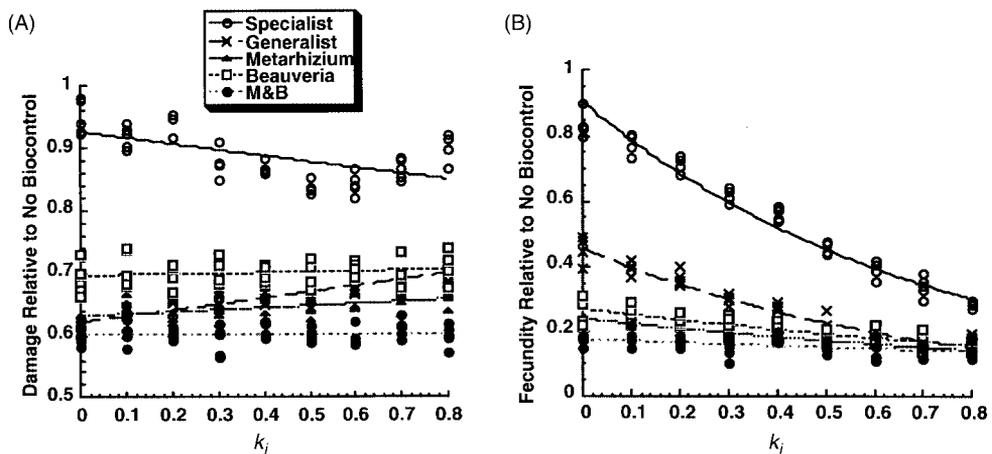


Fig. 9. (A) Crop damage and (B) locust fecundity relative to that without biocontrol versus k_i , the metabolic cost of infection per unit of fungal load (equation 3).

Climate and fungal strain

Simulations comparing healthy locust populations in hotter or cooler climates with equivalent locust populations in our standard environment predict that locusts consume approximately 5% more food and have 1% higher fecundity in a hotter climate, but 13% less food and 16% lower fecundity in a cooler climate. Adding in the fungal diseases to the different climates and comparing their effects relative to these simulations of healthy locusts suggest that all strains except the specialist provide worse control in a hotter climate than in our standard empirical climate and, conversely, slightly better control in a cooler climate

(Fig. 10A,B). The model results indicate that *B. bassiana*, in particular, is severely affected by the hot climate, since night-time temperatures are above the optimum for fungal growth. In contrast, the specialist does better in hotter than cooler climates because night-time temperatures in the hot climate now reach 27°C, which is the optimum for the fungus used in these simulations (Fig. 1).

DISCUSSION AND CONCLUSIONS

The behavioural model generates a number of predictions that are consistent with results from empirical investigations. In particular, the model captures very well the variability in pathogen performance reported from various laboratory and field studies under different environmental conditions. For example, the model predicts that *M. anisopliae* var. *acridum* should perform better than *B. bassiana* under conditions that allow active thermoregulation and that both pathogens should become less efficacious under hotter conditions. Empirical studies conducted in Canada indicate that *B. bassiana* can cause suppression of field populations of grasshoppers during cool overcast periods (Johnson and Goettel, 1993), but its effect is much less during hot sunny periods or when hosts are able to actively thermoregulate (Inglis *et al.*, 1997a,b). Similarly, Blanford and Thomas (1999a) showed that *B. bassiana* causes greater locust mortality in conditions in which host thermoregulation is restricted. On the other hand, studies on *M. anisopliae* var. *acridum* indicate that its development is less severely restricted under normal thermoregulatory conditions (e.g. Inglis *et al.*, 1997b; Blanford *et al.*, 1998), but that in particularly harsh environments with, for example, prolonged periods of high temperature during the day combined with

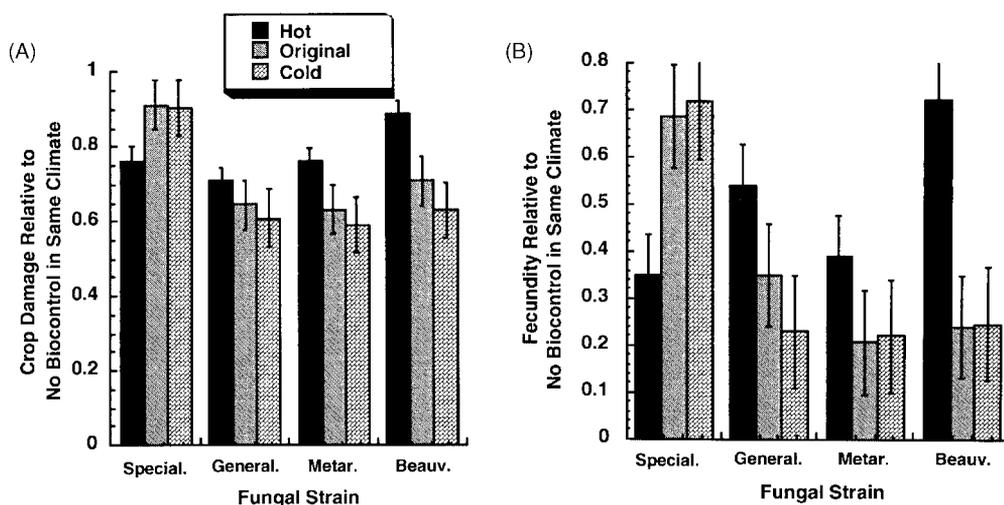


Fig. 10. (A) Crop damage and (B) locust fecundity by simulated populations exposed to different fungal strains in climates where the daytime temperature is 6°C above or below and night-time temperature is 3°C above or below the ambient temperatures used in the original simulation. Damage and fecundity are scaled relative to simulated populations without exposure to biocontrol in the same climate.

cool temperatures of 5–10°C at night, performance can be substantially reduced (Arthurs and Thomas, 2000). The model predictions concerning the outcome of co-infections with these pathogens also support empirical data. In a study in which grasshoppers were maintained at temperatures in excess of 35°C for approximately 6–8 h per day (a regime similar to our standard climate scenario in the model), co-infected hosts suffered the highest mortality, followed by hosts infected singly with *M. anisopliae* var. *acridum* and *B. bassiana*, respectively (Inglis *et al.*, 1997b).

One general output from the modelling study, therefore, is confirmation of the importance of environmental temperature and thermal behaviour/ecology of host and pathogen strain in mediating the outcome of infection. *M. anisopliae* var. *acridum*, *B. bassiana* and the hypothetical generalist fungus are predicted to provide better locust control than the hypothetical specialist fungus. Infection with the former three fungi may reduce crop damage by 35% and locust fecundity by 80% relative to a healthy locust population, if infection occurs early in the season. In the context of a biopesticide, this means early application is predicted to be better than spraying later when locusts are at an advanced stage of maturity. This is not simply because the different pathogen strains take a relatively long time to induce mortality, but also because there are indirect control benefits associated with changes in host behaviour that accrue over the season. This is illustrated particularly well with the specialist fungus, which is predicted to cause no direct mortality under our standard climate regime but still reduces damage and fecundity by 10% and 30%, respectively. Central to this prediction is that infected locusts reproduce earlier and at a smaller size than uninfected locusts. This prediction is supported by empirical data for brown locust (*Locustana pardalina*) (Arthurs and Thomas, 2000) and desert locust (*Schistocerca gregaria*) (Blanford and Thomas, 2001) infected with *M. anisopliae* var. *acridum*. Several studies have also reported a reduction in locust feeding associated with infection (e.g. Thomas *et al.*, 1997, 1998; Arthurs and Thomas, 2000), which again is an important behavioural consequence of infection with cumulative benefits in terms of overall control.

Extending the practical and biocontrol insights, the model illustrates very well how differences in thermal characteristics of individual pathogen strains may have marked effects on their impact in different environments. This suggests the potential for ‘climate matching’ and selecting strains in terms of thermal requirements of the host and its environment (Fargues *et al.*, 1997; Ouedraogo *et al.*, 1997). Recognition of the host ecology and behaviour is particularly important in this respect as host body temperature may differ considerably from ambient conditions; this applies to many insect species and not just strong behavioural thermoregulators such as those as studied here (May, 1979; Blanford and Thomas, 1999a,b). Moreover, although all the strains we examined had similar temperature optima, the differences in their upper and lower limits for growth and overall shapes of the thermal profiles led to considerable variation in efficacy. Perhaps of particular interest is how, under the variable temperature conditions considered in our simulations, the thermal generalist strain performed so much better than the thermal specialist. This cautions against evaluating the effects of pathogens under very restricted thermal conditions set around the *in vitro* thermal optima; this is commonplace in many applied and fundamental host–pathogen studies.

Returning to more fundamental insights, an interesting prediction from the model is that locusts can initiate variable behavioural fever responses depending on the pathogen strain. In our model, behavioural fever has therapeutic benefits and allows some infected hosts to reproduce, albeit at an earlier stage resulting in lower fecundity. However, fever has

direct costs through effects on development, feeding and susceptibility to predation. Here, these costs and benefits appear to be balanced by locusts having different degrees of fever depending on the thermal sensitivity of the pathogen. Hence, where the normal set point body temperature of the locusts exceeds the upper limit for growth of a pathogen, as it does for *B. bassiana* and the specialist, no behavioural fever is invoked. In contrast, *M. anisopliae* var. *acridum* and the generalist can grow at temperatures close to, or in excess of, normal body temperatures and locusts respond with behavioural fevers of varying magnitude. Evidence in support of this is provided by Blanford and Thomas (1999, unpublished data), who showed that locusts infected with *M. anisopliae* var. *acridum* exhibited a fever of 2–4°C (i.e. consistent in magnitude with that predicted by the model), whereas locusts infected with *B. bassiana* did not. Similarly, Adamo (1998) examined specificity of behavioural fever in the cricket, *Acheta domesticus*, in response to a range of parasites and only detected fever against a pathogen that was negatively affected by higher host temperatures. However, it remains unclear the extent to which the survival benefit of fever in insects results from a direct negative effect of temperature on the pathogen, or from indirect effects via changes in host immune response, or indeed from a combination of both. Studies on behavioural fever and therapy in crickets infected with rickettsia indicated a 6°C fever response, with body temperatures raised to a level where development of the pathogen was disrupted directly (Louis *et al.*, 1986). Studies on other arthropod taxa have reported a range of fever responses from 1.5 to 20°C (McClain *et al.*, 1988) and have suggested both direct and indirect effects of increased temperature on pathogenesis (see McClain *et al.*, 1988; Mitchell *et al.*, 1990; and references therein). Thus, we are uncertain whether the 5–6°C fever predicted in response to infection with the generalist fungus would actually be necessary for locusts to gain some therapeutic benefit; if the main effect of fever is enhanced immune function through, for example, increased production of haemocytes (Eslin and Prevost, 1998) or encapsulation rates (Konig and Schmid-Hempel, 1995), then an increase in set point temperature of just 2–4°C might be sufficient. Unfortunately, as there are no empirical data for locusts infected with a pathogen strain like the generalist, we cannot examine further the model prediction at this time.

Overall, the model adds to a growing body of empirical evidence highlighting the importance of temperature and thermal biology in host–parasite interactions. Given that neither the host nor pathogen biologies included in the model are particularly idiosyncratic, this is something that will apply to more species and scenarios than those explored here. From an applied perspective, the model identifies how host behaviour can dramatically affect the impact of an insect pathogen used in biocontrol. It also shows that there are important sub- or pre-lethal effects of disease, mediated via host behaviour, that contribute to control overall, and allows us to explore the impact of biocontrol under different scenarios. Given the generally poor and erratic performance of biopesticides reported under field conditions (Lisansky, 1997), and a heightened awareness of the potential negative environmental impacts of biocontrol (Simberloff and Stiling, 1996; Strong, 1997; Thomas and Willis, 1998), models such as ours could prove useful in prospective evaluation of biocontrol, identifying where and when different pathogens could be used to best effect and limiting unwarranted applications or introductions. From a more basic perspective, the model links insect behaviour with both population dynamic and evolutionary aspects of host–pathogen interactions. In so doing, it reveals novel mechanisms whereby costs of host defence may be tailored to individual diseases through selective expression of fever.

ACKNOWLEDGEMENTS

S.N.G. was supported by the United Kingdom NERC core funding to the Centre for Population Biology. This work was also performed under the auspices of the US Department of Energy by the University of California, Lawrence Livermore National Laboratory under Contract No. W-7405-Eng-48. The paper forms a part contribution to the EU-funded Environmentally Sustainable Locust Control Programme (ESLOCO – QLK5-CT-1999-01118) and the project ‘Development of biologically-based strategies for sustainable control of red locust in Central and Southern Africa’ funded by the United Kingdom Department for International Development (DFID) for the benefit of developing countries (R7818 Crop Protection Research Programme). The views expressed are not necessarily those of DFID. Discussion with S. Blanford is gratefully acknowledged.

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