# FUNGAL ECOLOGY AND ITS APPLICATION TO THE PRACTICAL USE OF MYCOINSECTICIDES

M B THOMAS
Leverhulme Unit for Population Biology and Biological Control, NERC Centre for Population Biology, Imperial College, Silwood Park, Ascot, Berks SL5 7PY, UK

S N WOOD
The Mathematical Institute, University of St. Andrews, North Haugh, St. Andrews, Fife KY16 9SS, UK

## ABSTRACT

The development of insect pathogens as control agents requires a comprehensive understanding of pest-pathogen dynamics if their full potential as biotic agents is to be realised. Aspects of the ecology of both pest and pathogen, however, can be difficult to measure. As a consequence, identification of the key factors which act to influence the dynamics of the pest-pathogen interaction can be difficult. In this context, mathematical population models can provide useful tools to focus research on the collection of the most relevant information and for developing and evaluating control strategies.

This paper describes the application of some simple population dynamic models to investigate the potential of a fungal entomopathogen for biological control of locusts and grasshoppers. The models are used to assess population fluctuations and reductions arising from biopesticidal applications of the pathogen under different scenarios and to determine what effects various aspects of the host-pathogen interaction have on the impact of a single application.

## INTRODUCTION

Diseases can have dramatic effects on populations of insects in both natural and agricultural settings (see Tanada & Kaya (1993) for numerous examples). However, despite their importance, the dynamics of insect-pathogen interactions remain relatively poorly studied. In particular, studies in which theoretical approaches are linked or supported with quantitative empirical data are rare. Thus, whereas biological control based on predators and parasitoids has available to it a large and established body of relevant theory, biological control using pathogens has only a limited theoretical basis. At the same time, because of environmental concerns and increasing insecticide resistance (recorded in over 500 insect species world-wide and increasing exponentially (Georghiou, 1990)), the opportunities for using conventional chemicals for insect pest control are becoming more and more restricted and the interest in the potential of insect pathogens for biological control, particularly as biorational pesticides, has been stimulated by recent developments in molecular biology and

biotechnology and is growing rapidly. Thus, efforts to improve understanding of host-pathogen dynamics and ecology are now both necessary and relevant.

This paper describes how ecological approaches are being applied in an on-going biological control programme which is aiming to develop a fungal-based biopesticide product for control of locusts and grasshoppers. The paper does not attempt to address insect-pathogen interactions from a broad theoretical perspective but instead, focuses on how basic ecological techniques are being used to help interpret and predict the impact of spray applications and, in more general terms, how this is contributing to a greater appreciation of both the similarities and the differences between chemical pesticides and their biological counterparts.

## The LUBILOSA programme

The LUBILOSA (LUtte BIologique contre les LOcustes et SAuteriaux) programme has been developing an oil-based biopesticide containing spores of the entomopathogenic fungus, *Metarhizium flavoviride*, for control of locusts and grasshoppers in Africa. This pathogen acts like a chemical pesticide through direct contact and a number of successful laboratory, semi-field and field tests have been conducted against a range of locust and grasshopper hosts under a variety of ecological conditions (e.g. Bateman *et al.*, 1993, Lomer *et al.*, 1993, Douro-Kpindou *et al.*, 1995, Kooyman *et al.*, in press). From these tests it has become clear that the host targets can be contacted by the pathogen via three possible routes. The first of these is from direct contact with the initial spray application. The percentage infection or mortality resulting from this route is governed largely by application techniques and physical environmental factors such as temperature, wind speed and vegetation structure; these factors also influence the initial efficacy of an application of a chemical insecticide. The second route of infection is via the spray residue. Once again application, formulation and environmental factors are important, through their effects on the persistence and spatial distribution of the pathogen propagules. However, in addition to these, biotic factors such as pathogenicity and natural survival of the pathogen are also important. These factors have their chemical analogues but will be influenced by environmental variables in different ways. Finally, horizontal transmission of the pathogen from individuals infected via the first two routes provides a third possible route of infection. The dynamics of this phase are governed by the factors which regulate natural host-pathogen interactions since infections result from natural pathogen delivery mechanisms. These clearly include a number of biotic factors relating to the specific life-history and behavioural traits of the host and the pathogen, as well as a range of abiotic factors which have a fundamental influence on the physical and biochemical processes involved in the host-pathogen interaction. These processes have very few chemical analogues.

Overall, therefore, if the consequences of an artificial application of pathogen on within- and between-season host-pathogen dynamics are to be investigated, the relative contribution of each of these separate routes of infection needs to be examined, taking into account both the biotic and abiotic aspects of the system. Since it is rather complicated (perhaps impossible) to do this through experimentation alone, examination of various aspects of the host-pathogen interaction is aided greatly by the use of population dynamic models.

## A BASIC HOST-PATHOGEN MODEL

A general framework for a model which captures the intra- and inter-generational events of a typical sahelian grasshopper system is presented below. Although the principles involved in this model are very simple, the mathematics necessary to describe events can, depending on assumptions, be complicated and are not discussed any further here. Instead, the paper focuses on the biological insights gained from the model and the model itself is described verbally. Those readers interested in further details of the model and its derivations are referred to Thomas *et al.* (1995, 1996a, 1997) and Wood & Thomas (1996).

Most sahelian grasshoppers have just one generation per year. Nymphs emerge during the wet season from eggs laid the previous year and pass through 5 or 6 instars to adulthood in 4-6 weeks. Reproduction and oviposition takes place over a similar period of about 4-8 weeks and then the adults die. Eggs remain in diapause in the soil throughout the dry season (a period of about 8 or 9 months) until the onset of the rains. During this period the eggs are protected from the pathogen. Therefore, any interaction between grasshopper and pathogen is restricted to the wet season (a period of approximately 120 days).

For the model then, the within-season component begins after egg hatch with a population of susceptible grasshoppers of density $H$. A spray event results in a proportion of these becoming infected. This proportion depends on spray efficiency, $k$, which is a composite parameter describing the direct spray contact rate, $d$, and the infectivity of the spray residue, $r$. After a number of days (the number actually depends on the host species, the virulence of the pathogen isolate and environmental conditions) these insects die, produce fresh pathogen spores and thus act as new sources of infection. Each individual cadaver has a defined infectivity profile which varies as spores are released and the body parts break up and decay but the overall rate of infection of susceptible hosts is determined by the summed infectivity, $i$, of the cadaver population. This latter term is also a composite function that assumes that the instantaneous risk of infection to a given susceptible host is a function of cadaver density and their individual ages at that instant. Thus, $i$ contains elements of both the transmission coefficient, (i.e. the constant proportion of contacts between hosts and pathogen that actually result in transmission, $\beta$), and the dynamics of the free-living pathogen population (i.e. the transition rate for change in infectivity as individual cadavers age, $c$).

For the interval between infectious seasons, it is assumed that the host population grows at a finite rate of increase, $F$, where those individuals available to breed are the fraction of susceptible hosts that survived the epidemic. The between-season changes in host population dynamics can also include an immigration term, $R$, which represents the mean number of grasshoppers that arrive in an area independent of density last year due to redistribution between feeding and oviposition sites. Furthermore, the model can allow for carry over of the pathogen between wet seasons by scaling infectivity of the cadaver pool by a constant proportion, $g$, representing pathogen survival.

This basic form of the model omits all sources of mortality other than the pathogen; a conservative assumption when asking questions about how effective control is likely to be.

The model also assumes a proportional mixing form for the transmission process, a standard assumption in many host-pathogen models.

## MODEL SIMULATIONS

### Effects of spray residue on impact of a spray application

Having formulated a basic model it is now possible to use this to explore the consequences of spray applications under different scenarios and examine how the various routes of infection combine to influence grasshopper population densities. To begin with, just the events following the initial spray application are examined, highlighting the contribution of the spray residue to overall mortality. In order to do this, it is necessary to set some basic parameters for the model and define the infectivity of the spray residue, $r$.

Numerous studies in the LUBILOSA programme have revealed that the infectivity of the spray residue typically follows a negative exponential decay with a half-life of between 2-8 days (Jenkins & Thomas 1996, Thomas *et al.* 1996a, 1997, Kooyman *et al.* in press). It is worth noting that this persistence is considerably greater than expected from laboratory investigations which reveal that only 1h of exposure to simulated tropical solar radiation can dramatically reduce conidial viability and a single day of direct sunlight causes complete inactivation (Moore *et al.* 1993). Thus under natural field conditions, spores appear to remain active in protected positions in the environment for far longer than might be expected. For the following examples then, the spray residue is considered to have a half-life of between six and seven days. The expression used in the model to describe this risk of infection from the spray residue ($r$) across time (t) is given by $r = 0.156 \exp(-0.102t)$. This describes a negative exponential and represents an empirically measured estimate obtained from field trials against the rice grasshopper (*Hieroglyphus daganensis*) in north Benin (see Thomas *et al.* 1997 for details). Using this estimate, the model is used to investigate the contribution of the spray residue to total mortality within a season and to examine what effects this has on long-term control (Figure 1). For the sake of this exercise it is assumed that the grasshopper population is sprayed whenever it exceeds a threshold density of 10 grasshoppers/$m^2$ (there are no good estimates of economic threshold densities for Sahelian grasshoppers; 10/$m^2$ is an arbitrary figure representing intermediate to high grasshopper densities). It is also assumed that spraying only occurs once a year and that initial spray efficiency is low. This is done here so that the potential contribution of the spray residue can be clearly seen, although direct spray contact rates can indeed be very low under real field conditions. Other parameter values for the model are given in Table 1.

The model output in Figure 1 and subsequent figures shows the host population trajectory over 50 generations with the time between seasons removed to shorten the time series for presentational purposes. The figure reveals that although the spray hit rate is low (just 15%), total mortality following a spray application is approximately 80%. This causes the population to crash dramatically and thus provides a substantial contribution to the impact of a spray application. Although not discussed further here, this within-season prediction has been validated for a number of field trials, confirming the importance of residual pick-up of spores as a major route of infection (Thomas *et al.* 1996a, 1997). However, in spite of this impact the biopesticide fails to provide any long-term control with host densities exceeding

the spray threshold nearly every year. Thus, under these model assumptions the biopesticide acts just like a chemical pesticide inducing a standard level of density independent mortality.

Table 1. List of parameters used in the model. Parameter values are taken from Thomas *et al.* (1995, 1996a,b, 1997) and Wood & Thomas (1996).

| Parameter description | Value |
|---|---|
| Transmission coefficient (β) | 0.4 |
| Transition rate for change in infectivity of cadavers ($c$) | 0.11 |
| Risk of infection from the spray residue ($r$) | 0.156 exp(-0.102t) |
| Time to death following infection | 12 days |
| Density of healthy hosts before spraying | $10/m^2$ |
| Finite rate of increase ($F$) | 5 |
| Between-season pathogen survival rate ($g$) | 0.05 |
| Annual immigration of healthy hosts ($R$) | $0.1/m^2$ |
| Spray action threshold | $10/m^2$ |
| Direct spray contact rate ($d$) | 0.15 |
| Duration of season after spraying | 90 days |

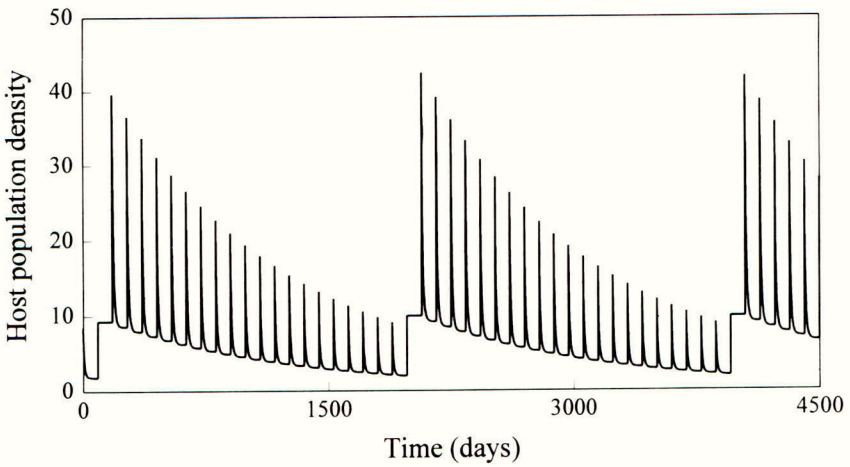


Figure 1. Predicted change in grasshopper population density through time following applications of the biopesticide. In this example, the biopesticide acts through direct contact and residual pick-up of spores only, with no horizontal transmission. Parameters for the model and details of the spray regime are given in Table 1 and the main text.

Effect of horizontal transmission on impact of a spray application

Having examined the effect of the spray residue, the contribution of horizontal transmission of the pathogen to overall mortality following a spray application is now examined. In order to examine just the effects of horizontal transmission, it is assumed that residual pick-up is zero and the initial spray application simply acts to provide a pulse of infected individuals at the beginning of the season. To explore the effect of further cycles of infection following this introduction it is now necessary to add in estimates for the transmission coefficient, $\beta$, and the transition rate for movement of cadavers between infectious stages, $c$. These have been estimated empirically for a number of grasshopper species in a variety of habitats. For the purpose of continuity, the parameter values used here are once again for *H. daganensis*. Full details of the experiments conducted to obtain these estimates are given in Thomas *et al.* (1995). For the current purposes it is sufficient to note that $\beta$ and $c$ were estimated as 0.4 and 0.11, respectively. Other parameter values are the same as in Table 1.

As with the spray residue, the model is used to examine the total mortality of grasshopper populations when sprayed with the biopesticide (Figure 2). This figure illustrates two important points. First, the biopesticide acts in a density dependent manner. This results in high density populations suffering proportionately greater mortality than low density populations. This is indicated by the fact that the high density populations (represented by the high peaks) crash abruptly and are always followed by low density populations (low peaks); thus high density conditions promote a larger epidemic. Second, the effect of horizontal transmission is very strong and even with the low spray contact rates, mortality is extremely high by the end of the season with the result that spray frequency falls to one in every four years. Interestingly, however, for the realistic parameter ranges determined empirically for this model, the pathogen fails to control the grasshoppers in a sustainable manner. Although not shown here, whatever proportion of the pathogen is allowed to persist from one year to the next, either the host or the pathogen is effectively driven to extinction. Since host extinction is most unlikely in practice, what this really means is that sustained "classical" biological control is unlikely to succeed using this pathogen, at least under these model assumptions. Nonetheless, the results of this study do highlight the possibility of exploiting the biological properties of relatively ineffective indigenous pathogens to develop biopesticides that act in a density dependent manner. This possibility has rarely been considered in the development or strategic use of entomopathogens in biological control.

Unlike the residual infection example, the predictions from the simple horizontal transmission model have yet to be fully validated in the field. A number of studies have suggested secondary cycling of the pathogen following spray applications (e.g. Baker *et al.* 1994, Thomas *et al.* 1997) but a major effect on overall mortality as indicated here has yet to be demonstrated. One possible reason for this is that some of the basic model assumptions may be flawed. In order to identify any errors in model structure and formulation, studies on a range of factors such as the relationship between pathogen and host density and the rate of infection, sub-lethal and behavioural responses to infection, and the fate of infected hosts and cadavers in the field, are currently underway. However, one of the major differences between the basic model and events that follow a real spray application is the omission of any sequential effects of the different routes of infection after spraying. That is, it has been shown already that residual pick-up of spores can itself result in high overall mortality and may

continue to have an impact for some time after spraying. Under these circumstances, the spray application does more than just provide a small pulse of infection. How this influences secondary cycling and which of these routes of infection is the most important in terms of practical control is unclear. This is examined in the following section.

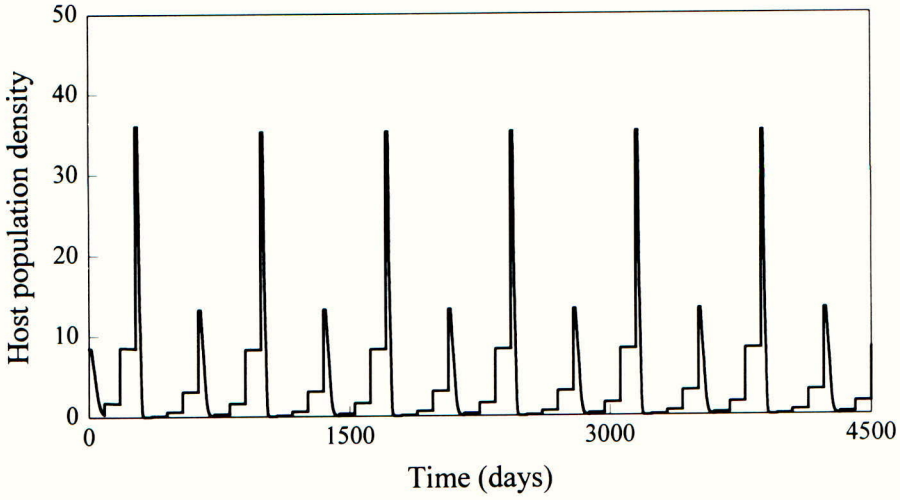


Figure 2. Predicted change in grasshopper population density through time following applications of the biopesticide. In this example, the biopesticide spray acts to create a small pulse of infection at the beginning of the season and subsequent mortality results from horizontal transmission. Other details are the same as for Figure 1.

The combined effects of residual infection and horizontal transmission on the impact of a spray application

The basic residual pick-up and horizontal transmission models can be combined easily into a single multi-year model. The output from this combined model is shown in Figure 3. This shows combined qualities of previous outputs, with both rapid reduction in populations after spraying and reduced spray frequency. The overall conclusion from this is that residual pick-up appears to kill more grasshoppers because it acts first while numbers are still high, but secondary cycling clears up those remaining. Thus, although residual pick-up produces rapid results, it is secondary cycling that will drastically reduce the host population next year (at least if a large enough area is treated) and which in turn, contributes to better overall control reducing spray frequencies and restricting population peaks. This result is important in any context in which the choice of pathogen strain or formulation involves a trade-off between direct and residual kill rates, and the potential for secondary cycling. For example, a trade-off between virulence and pathogen reproduction (i.e. spore production) has been noted for a number of fungal isolates tested in the LUBILOSA programme. Similar relationships have also been identified for certain viruses. Thus, although high virulence may be a desirable trait (virulence is often the principal criterion for isolate selection), selecting isolates on the

basis of this factor alone may have unforeseen consequences for the population dynamics of the host-pathogen interaction and overall pest control.

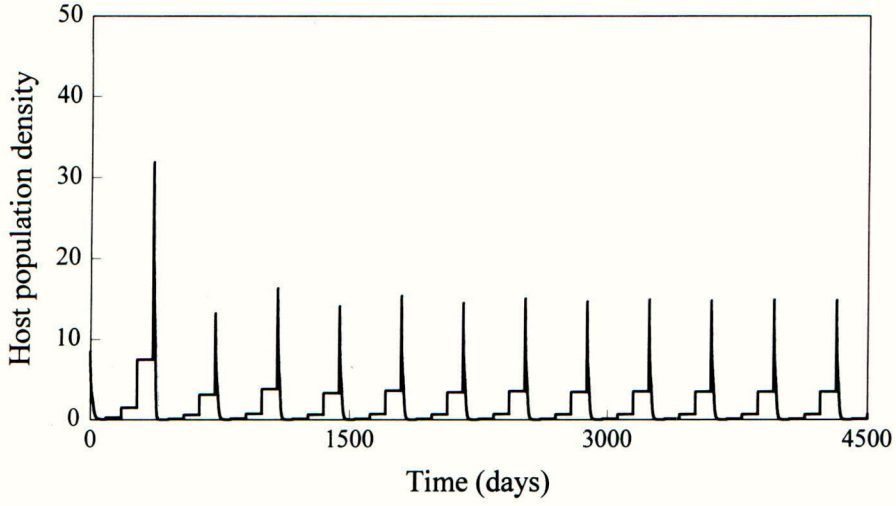


Figure 3. Predicted change in grasshopper population density through time following applications of the biopesticide when both residual pick-up of spores and horizontal transmission occur. Other details are the same as for Figure 1.

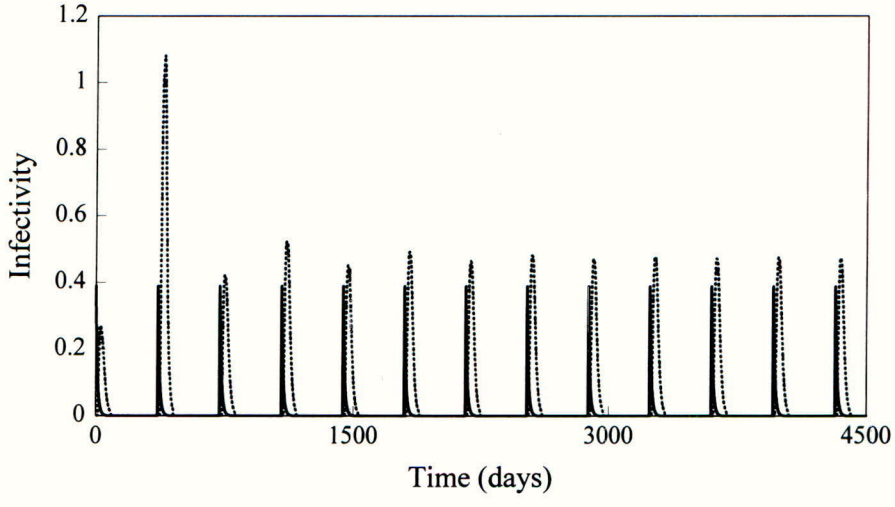


Figure 4. The relative levels of infectivity in the environment caused by the spray application and residual pick-up of spores (solid line) and subsequent horizontal transmission. Further details are given in the main text.

Further detail is revealed by examining the relative levels of infectivity in the environment provided from the different routes of infection (Figure 4). This confirms that the spray residue

acts first, is short lived and acts in a density independent manner. The pattern of infectivity from horizontal transmission then follows and because of cycling, lasts across the season and varies with host density. Hence, it can be seen that under realistic conditions when direct hit and residual pick-up have a high initial impact, secondary cycling still has an important, albeit subtle role to play. Moreover, secondary cycling is not likely to be apparent until late in the season and so short-term field trials which run for just part of the season (which is a standard feature of nearly all trials to date) are not best suited to demonstrating its effects.

## DISCUSSION

The study of insect-pathogen dynamics and the development of insect pathogens as biocontrol agents is an exciting and, in some ways, neglected area of research. This paper has focused on the development and application of insect-pathogen models within a particular biocontrol programme with the aim of demonstrating the potential for utilising population dynamic approaches to address real problems in applied pest management. Here, for example, the models provide a tool for interpreting patterns of mortality following spray applications and evaluating the relative importance of different routes of infection. More than that, since the model frame work and biology are appropriate to many seasonal systems, the work presented here provides some novel insights relevant to development of insect pathogens as biocontrol agents in general. First, because of the biological nature of the active ingredient, a biopesticide may have a density dependent component to its action. As stated above, this possibility has rarely been considered in the development of biopesticides. Identifying this fundamental difference between biopesticides and conventional pesticides could provide new opportunities for the use of pathogens in biological pest control. Furthermore, it could have significant consequences for the economics of biopesticide use. This is seen in the combined model where although in absolute terms, secondary cycling of the pathogen appears to have little impact, its effect on reducing the frequency of spray applications is most pronounced. Related to this then, in order to fully evaluate the efficacy of a biopesticide, it is necessary to understand that like any living control agent, the effectiveness of a pathogen depends not just on its capacity to kill pests but also its capacity to reproduce on pests and thereby continue and compound its killing action. The interaction between these functional and numerical components of biopesticide activity can be subtle and may not be apparent from short-term field studies. This highlights the need for an increased appreciation of both the similarities and the differences between biopesticides and chemical pesticides and cautions against considering biopesticides simply as analogues of chemical pesticides with slow acting active ingredients. Given the need to change this traditional pesticide "mind-set", this also confirms the role of population ecology as fundamental to the future development and adoption of biopesticide technologies.

## ACKNOWLEDGEMENTS

LUBILOSA is a research programme on the biological control of locusts and grasshoppers executed by the International Institute of Biological Control, of CAB INTERNATIONAL; the International Institute of Tropical Agriculture Plant Health Management Division; AGRHYMET-CILSS, Centre pour Agronomie, Hydrologie, Meteorologie of the Comité

Inter-État de Lutte contre la Secheresse au Sahel and; Deutsche Gesellschaft für Technische Zusammenarbeit (GTZ) GmbH. LUBILOSA is funded by the governments of Canada (CIDA), Britain (ODA), the Netherlands (DGIS) and Switzerland (SDC).

## REFERENCES

Baker, G L; Milner, R J; Lutton, G G; Watson, D (1994) Preliminary field trials on control of *Phaulacridium vittatum* (Sjöstedt) (Orthoptera: Acrididae) populations with *Metarhizium flavoviride* Gams & Rozsypal (Deuteromycotina: Hyphomycetes). *Journal of the Australian Entomological Society* **33**, 190-192.

Bateman, R P; Carey, M; Moore, D; Prior, C (1993) Oil formulations of entomopathogenic fungi infect desert locusts at low humidities. *Annals of Applied Biology* **122**, 145-152.

Douro-Kpindo, O K; Godonou, I; Houssou, A; Lomer, C J; Shah, P A (1995) Control of *Zonocerus variegatus* with ULV formulation of *Metarhizium flavoviride* conidia. *Biocontrol Science and Technology* **5**, 131-139.

Georghiou, G P (1990) Overview of insecticide resistance. In: *Managing Resistance to Agrochemicals*. M B Green, H M LeBaron & W K Moberg (eds), Washington, DC: American Chemical Society, pp. 18-41.

Jenkins, N E; Thomas, M B (1996) Effect of formulation and application method on the efficacy of aerial and submerged conidia of *Metarhizium flavoviride* for locust and grasshopper control. *Pesticide Science* **46**, 299-306.

Kooyman, C; Bateman, R P; Langewald, J; Lomer, C J; Ouambama, Z; Thomas, M B (in press) Operational-scale application of entomopathogenic fungi for control of Sahelian grasshoppers. *Proceedings of the Royal Society of London* B.

Lomer, C J; Bateman, R P; Godonou, I; Kpindou, D; Shah, P A; Paraiso, A; Prior, C (1993) Field infection of *Zonocerus variegatus* following application of an oil-based formulation of *Metarhizium flavoviride* conidia. *Biocontrol Science and Technology* **3**, 337-346.

Moore, D; Bridge, P D; Higgins, P M; Bateman, R P; Prior, C (1993) Ultra-violet radiation damage to *Metarhizium flavoviride* conidia and the protection given by vegetable and mineral oils and chemical sunscreens. *Annals of Applied Biology* **122**, 605-616.

Tanada, Y; Kaya, H K (1993) *Insect Pathology*. San Diego: Academic Press.

Thomas, M B; Wood, S N; Lomer, C J (1995) Biological control of locusts and grasshoppers using a fungal pathogen: the importance of secondary cycling. *Proceedings of the Royal Society of London* B **259**, 265-270.

Thomas, M B; Langewald, J; Wood S N (1996a) Evaluating the effects of a biopesticide on populations of the variegated grasshopper, *Zonocerus variegatus*. *Journal of Applied Ecology* **33**, 1509-1516.

Thomas, M B; Gbongboui, C; Lomer, C J (1996b) Between-season survival of the grasshopper pathogen *Metarhizium flavoviride* in the Sahel. *Biocontrol Science and Technology* **6**, 569-573.

Thomas, M B; Wood, S N; Langewald, J; Lomer, C J (1997) Persistence of biopesticides and consequences for biological control of grasshoppers and locusts. *Pesticide Science* **49**, 47-55.

Wood, S N; Thomas, M B (1996) Space, time and persistence of virulent pathogens. *Proceedings of the Royal Society of London* B **263**, 673-680.