Biological Control of Locusts and Grasshoppers

Edited by C. J. Lomer and C. Prior, International Institute of Biological Control

In the period 1985–8 rains over much of Africa after a period of prolonged drought triggered the development of plague populations of grasshoppers and locusts. Countries from Senegal and Mauritania in the west to Iran in the east were affected. The use of costly and sometimes environmentally hazardous insecticides did contribute to the subsequent control of the problem, but highlighted the need for a reassessment of locust control methods. As a result, a collaborative programme was initiated in 1989 and undertaken by the International Institute of Biological Control of CAB International (CABI–IIBC), the Biological Control Program of the International Institute of Tropical Agriculture (IITA–BCP) and the Department of Crop Protection Training of the Permanent Inter-State Committee for Drought Control in the Sahel (CILSS–DFPV)). Its main objective is to show the feasibility of killing locusts and grasshoppers using an oil-based formulation of a fungal pathogen.

This volume contains the proceedings of a workshop held at Cotonou, Republic of Benin, from 29 April to 1 May 1991, and organized as part of this collaborative programme. The book consists of six parts: (1) summaries of activities of organizations and country reports: (2) agents for biocontrol; (3) exploration and characterization; (4) mass production, application and formulation; (5) biology, ecology, field experimentation and environmental impact; and (6) closing session. Topics peripheral to the research programme, as well as central to it, are addressed in the book. It will therefore attract a wide potential readership among those concerned with insect pest management in general, as well as locusts and grasshoppers and the potential for mycopesticides in particular.

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Proceedings of a Workshop held at the International Institute of Tropical Agriculture, Cotonou, Republic of Benin, 29 April–1 May 1991

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C·A·B International

in association with

International Institute of Tropical Agriculture

C·A·B International
Wallingford
Oxon OX10 8DE
UK

Tel: Wallingford (0491) 32111
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Oyo Road
PMB 5320
Ibadan
Nigeria
Tel: (234 22) 400 300
Telex: TDS IBA NG 31417 TROPIB NG

in association with

International Institute of Biological Control
(an Institute of C·A·B International)
Silwood Park
Buckhurst Road
Ascot
Berkshire SL5 7TA
UK
Tel: 0344 872999
Telex: 9312102255 BC G
Telecom Gold/Dialcom: 84: CAUG15
Fax: 0344 872901

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Foreword
Locust control – cause for concern

Lukas Brader

Director General, International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria, former Director of the FAO Emergency Centre for Locust Operations (ECLO), Rome, Italy.

Locusts have caused problems to man's agricultural efforts and well being ever since the first historical records. During the 1930s to 1960s, a period of sustained locust attack, research by a number of institutions including the Anti-Locust Research Centre led to the development of effective control strategies. These were based on effective monitoring and forecasting and using mainly dieldrin, primarily to lay down persistent barriers to control hopper bands.

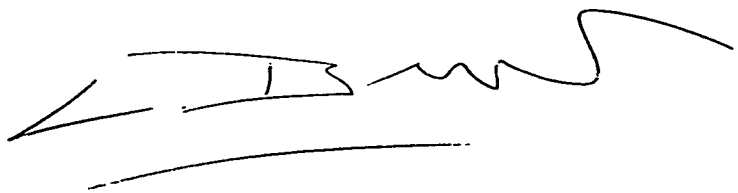
The period from 1960s to 1985 was a remarkably quiet time for locusts, the longest inter-plague period known. Regretfully, locust surveillance, control and research were allowed to run down at the same time. Also during this period, the rise of environmental concerns led to the banning of many persistent organochlorine insecticides including dieldrin. Another development of note was that legislation adopted in the USA (and later elsewhere) arising from concerns about dumping of toxic chemicals in developing countries, made it impossible for countries in receipt of US aid to carry on using dieldrin.

Thus when locust plagues did recur in 1985, not only were organizations charged with control and monitoring unprepared, they had also been deprived of their most powerful weapon for hopper control. The less persistent organophosphates, carbamates and more recently the synthetic pyrethroids used in place of dieldrin made hopper control more complicated because they had to be sprayed over the whole infested area and had to be applied much more frequently. As such, they have led to undesirable environmental effects of their own. However, for the control of adults, these new insecticides proved very useful.

The challenge is now to develop effective control measures and strategies, including biological control methods, which will have reduced environmental effects, lower costs and equal or greater effectiveness than

existing methods. Problems in application will always remain; the huge extent of the locust recession areas, and their inaccessibility will make locust control always a difficult job. Models predicting outbreaks are still unreliable and despite the advances made in the use of satellite technology, we still rely on ground reports for most of our information.

The ideas presented in this volume give some indication of the new possibilities for control offered by biological means.



Preface

This volume contains the proceedings of a workshop on biological control of locusts and grasshoppers, held at Cotonou, Republic of Benin, from 29 April to 1 May 1991. The workshop was funded by a grant from USAID, and organized as part of the joint IIBC/IITA/DFPV collaborative research programme on the biological control of locusts and grasshoppers. Details of the programme are given in the relevant section of this book. The programme is funded by CIDA, USAID, ODA and DGIS.

The workshop was originally planned as a working forum to discuss subjects specifically relevant to the programme; however, the response to invitations sent out was so great that the concept had to be expanded to include a variety of peripheral topics. Nevertheless, useful discussion took place, some of which is summarized here.

The talks presented at the workshop have been edited, translated from French where necessary, and refereed in the case of all but the first section. The first section (I) consists of summaries of the activities of various organizations involved in locust and grasshopper control and some country reports.

The remaining sections cover technical aspects of locust and grasshopper biocontrol; (II) agents for biocontrol; (III) exploration and characterization; (IV) mass production, application and formulation; and (V) biology, ecology, field experimentation and environmental impact. An edited precis of the discussions in the closing session (VI) is also presented. We invited speakers who have had practical experience in the use of fungi for biocontrol in a variety of situations; in sugarcane in Brazil, in forests in Switzerland, in grassland in Australia. As the reader will note, there seems to be a great mood of optimism concerning the possibilities for control using fungi, and we hope that the information presented here will encourage others to try for themselves.

A French version will be produced in due course.

Acknowledgements

The conference organizers greatly appreciate the consideration and co-operation shown by the government of our host country, the Republic of Benin.

We would like to thank all the speakers who presented papers and also those who contributed from the floor to the discussions. Speakers and referees provided their written texts promptly and we thank them for enabling us to produce these proceedings with the minimum of delay.

Jacob Quaye and Samson Acheyompong (IITA-BCP) provided invaluable logistical support.

We are very grateful to Annette Greathead for meticulous copy editing, to Clare Lord, Oscar Hounvou and Carole Moudachirou for simultaneous translation during the meeting, and to Cecile Lomer who collated the manuscripts and translated the French texts.

The workshop was financed out of grant AFR-0517-G-IN-9096-00 from USAID and we are grateful to Dr W. Knausenberger of USAID (Africa Bureau) and to all our donors for their material support and encouragement.

Welcome address

Representative of the Minister for National Education

The Representative of the Minister for Rural Development and Cooperative Action, Representatives of International Organizations, Representatives of Donor Agencies, The Director of the International Institute of Biological Control, The Representative of the International Institute of Tropical Agriculture, The Director of the Biological Control Centre for Africa, Representatives of National Plant Protection Organizations, Honoured Guests and Research Scientists, Ladies and Gentlemen:

I am delighted to welcome to Cotonou scientific experts and all those partners in scientific development who have kindly come to Africa to the free territory of Benin to exchange views and experiences in the field of biological control of locusts and grasshoppers, which are major pests of crops in the tropical and Mediterranean zones of Africa and also world-wide.

Your acceptance of this invitation from IITA and IIBC demonstrates without doubt, the interest which the international community shows in the vast regions of Africa which are subject to invasions by grasshoppers. Those of you here in Cotonou, are, by your very presence, lending your support to the new biological control project run jointly by IITA and IIBC at the IITA research station at Abomey-Calavi, which is trying to curb this scourge. This is a project in which the Beninois government and people, and all African farmers have a great deal of hope.

In fact, despite strenuous financial efforts by donors, it is an inescapable fact that chemical means of control are not sufficient to give lasting respite from the pressure of locusts and grasshoppers, which jeopardise the best laid plans for ensuring adequate food supplies every year, in every affected region. Besides this, when one knows that the solution offered by chemical pesticides is compromised by the environmental effect of the chemicals entering the food chain and affecting the environment, the

importance of biological control in this domain can be clearly appreciated.

Ladies and gentlemen involved in scientific research:

Let me tell you that all African farmers, people concerned with socio-economic development and the populations of both town and country await with impatience new technical packages which your work will bring to National Plant Protection programmes and to farmers. I must remind you that this workshop gives you a good opportunity to examine critically your results, your methods and the obstacles which impede the rapid development of biological control techniques for locusts and grasshoppers. This forum also gives you a chance to work out the most effective strategies for acquiring a better grasp of the pathology of these insects, production of pathogens, application and field trials, related ecological problems and regional and international co-operation.

In this respect, this workshop – the first of its kind – is only the first step in a long process, for there is still so much work to be done, the stakes are so important and the subject so interesting. Here we should thank the donors for making their funds available and for their solicitude. I am convinced that they still believe in the cause and will spare no effort to hold regular meetings of this kind where scientists from different disciplines and sectors can meet to agree upon effective action.

Now I shall address myself particularly to the representatives of National Plant Protection organizations.

In the process of developing appropriate biological control techniques against locusts and grasshoppers, it seems to me that you have a particularly heavy responsibility, since you are at the same time those who must act and those who benefit.

Your active contribution is required in the exploration phase and in the characterization of diseases of local Orthoptera, in identifying fungi and other natural pathogens of the insects and in defining optimum rearing conditions. As beneficiaries, you are under an obligation to master the techniques for *in situ* experimentation, methods of evaluating the impact of biological pesticides on the environment and the norms and methods of applying formulations in the field. You will not be able to fulfill your role without interesting the powers that be whom you must inform, and enlisting the co-operation of the populace whom you must impress and whose confidence you must gain.

The Representative of the Minister for Rural Development and Cooperative Action, Ladies and Gentlemen:

The organization of a forum at this level involves important undertakings and this is why I should like to thank all those who have been kind enough to lend their support.

I should like to mention USAID, GTZ, UNDP, USDA/APHIS.

My thanks also to IITA and IIBC who have made this workshop possible.

Once more, I thank national, international and non-governmental organizations and institutes, from far or near, who have all contributed to the present gathering, and all those research scientists who agreed to come here and take part in this meeting.

Wishing you the very best success in your work, I declare open, on this day Monday 29 April at Cotonou, the Workshop on Biological Control of Locusts and Grasshoppers.

Thank you.

Keynote address
Biological control as a potential tool for locust and grasshopper control

D.J. Greathead

International Institute of Biological Control

In 1985, near normal rains over much of Africa for the first time after a prolonged period of dry years triggered the development of widespread infestations of grasshoppers in West Africa; principally the Senegalese grasshopper, *Oedaleus senegalensis* (Krauss) in the northern part but also several other species further south. There were also simultaneous upsurges in the populations of the four species of locusts of major importance in tropical Africa for the first time in 50 years. A year later desert locust, *Schistocerca gregaria* (Forskål), numbers had reached plague proportions and threatened countries throughout its invasion area in Africa, the most alarming development since the last major plague collapsed in 1963. Breeding conditions remained favourable through 1986–88. During this period countries from Senegal and Mauritania in the west to Iran in the east were affected by swarms and hopper bands (Brader, 1988; OTA, 1990). Also, desert locust swarms crossed the Atlantic confounding the predictions of the experts that this could not happen (Ritchie & Pedgley, 1989). However, the desert locust plague collapsed in 1989 after failure of the rains during the winter breeding season. Unfortunately, because of the unprecedentedly long recession period, national and regional locust control organizations had been allowed to run down and were short of resources and expertise to combat the plague.

Locust control had depended on ultra-low volume (ULV) application of dieldrin for many years following research during the 1949–63 plague. This technique was particularly valuable for controlling hopper bands because a grid of sprayed swaths could be laid down which persisted throughout a season and killed any bands stopping to feed as they crossed them. Dieldrin was also valuable for swarm control but had begun to be replaced by less persistent organophosphates such as malathion. However,

concern about the effects of persistent pesticides on the environment and the banning of dieldrin in many countries at the end of the 1970s, including countries affected by locusts, forced the abandonment of this product. One consequence is the need to apply repeated blanket sprays over large areas to achieve the same results as formerly provided by single applications of dieldrin over a fraction of the area (Brader, 1988; OTA, 1990).

The donor community responded to the crisis by contributing some US$275 million towards locust and grasshopper control mainly in the Sahel and north-west Africa (Table 1). As a result of this donor assistance and their own resources, about 4.6 million ha of land in 10 countries received aerial or ground insecticide treatments in 1986–7 against locusts or grasshoppers, and 10 million ha were sprayed in 1988. Some 13 million l of insecticides were applied, mostly malathion and fenitrothion. The large-scale repeated application of insecticides raised concern about the possible impact on the environment as well as on human health (OTA, 1990). A pilot study on behalf of the FAO measured the impact of organophosphate insecticides and an insect growth regulator on insects, birds, aquatic invertebrates and fish. Adverse effects were recorded on all these categories of non-target organisms, including families containing natural enemies of locusts and grasshoppers, by one or more of the test chemicals (Everts, 1990).

Clearly, a reassessment of locust control methods was required and the FAO convened a desert locust research conference entitled *Defining Future Research Priorities* in October 1988. *Inter alia* the conference considered alternatives to chemical pesticides for locust control and 'expressed its great interest in the ideas' put forward by IIBC for research on the possibility of developing a biological pesticide (FAO, 1989). These ideas (Prior & Greathead, 1989) were developed into a proposal for a research programme by the International Institute of Biological Control (IIBC) of CAB International. This was eventually funded by the Canadian International Development Agency (CIDA), the Directorate General for Development Cooperation of the Netherlands (DGIS), the Overseas Development Administration of the UK (ODA) and the United States Agency

Table 1. Donor assistance to locust and grasshopper control programmes, 1986–89 in US$.

Bilateral donors	192,060,553
Multilateral donors	60,545,175
NGOs	2,455,460
Additional contributions	20,000,000
Total	**275,061,188**

From OTA (1990).

for International Development (USAID). It began in 1989 as a collaborative three year programme being undertaken by IIBC, the International Institute of Tropical Agriculture (IITA) and the Département de Formation en Protection des Végétaux (DFPV) in Niger.

This programme, entitled *Biological Control of Locusts and Grasshoppers*, has as its essential objective demonstration of the technical feasibility of killing locusts and grasshoppers using an oil-based formulation of a fungal pathogen, and involves the following components:

1. exploration for pathogens
2. evaluation of these pathogens as potential active ingredients of a biological pesticide
3. population ecology of locusts and grasshoppers in relation to the effects of pathogens
4. formulation of pathogens for ULV application
5. application of pathogens to demonstrate their efficacy as a control agent
6. development of mass production methods for pathogen spores (IIBC, 1990)

Although the programme had its origin in the concern generated by the desert locust plague and the massive application of pesticides applied to control it, the objective includes grasshoppers. The reasons for this are very important. Firstly, analysis of the desert locust plague by the US Congress Office of Technology Assessment (OTA), based partly on a questionnaire sent to individuals and organizations concerned with locust control, concludes that grasshoppers may be responsible for 5–18% of crop loss in each year (OTA, 1990) whereas locusts do not cause more than 4% loss even in peak plague years (Bullen, 1972). Thus, grasshoppers constitute a continuing and more serious threat every year than locusts during plague years only. Secondly, because of the intermittent nature of locust plagues, a product used for locust control and which cannot be stockpiled is most unlikely to continue to be produced during inter-plague periods unless it has other uses. Therefore, a product which is used to control grasshoppers will be in continuous production so that all that is needed if there is an upsurge in locust activity will be an increase in production to meet the emergency.

This address has outlined the circumstances leading to the initiation of the locust and grasshopper biological control programme and its rationale. We, the collaborating institutes, believe that we have made good progress in meeting the objectives of the programme and are proud of our achievements so far. This conference has been convened to discuss progress, obtain the views of specialists in the various disciplines involved in its execution, and most important, to inform and solicit guidance from the countries and organizations which will eventually have the opportunity to

use any product which is developed. Steps are being taken on behalf of the donors to ensure, by means of protective patents, that the results will be freely available to affected countries so that they can benefit from setting up their own production plants, and as far as possible make use of cheap locally available materials to manufacture the product.

The conference has been planned in such a way as to provide an authoritative review of each main topic by a specialist, followed by a discussion led by expert panellists during which it is hoped that you will contribute. The formal reviews, contributed papers, and a summary of the discussions and conclusions will be published to provide a statement of the state of the art for biological control of locusts and grasshoppers in the tropics. I look forward to an informative and stimulating few days, and in advance would like to thank all those whose efforts have made this conference possible and hopefully a big success.

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The IIBC/IITA/DFPV collaborative research programme on the biological control of locusts and grasshoppers

C. Prior, C.J. Lomer

International Institute of Biological Control.

H. Herren, A. Paraïso

International Institute of Tropical Agriculture.

C. Kooyman, J.J. Smit

Département de Formation en Protection des Végétaux.

Introduction

Locusts (particularly the desert locust, *Schistocerca gregaria* (Forskål)) and grasshoppers (*Oedaleus senegalensis* (Krauss), *Kraussaria angulifera* (Krauss) and many other species) are the most serious insect pests of agriculture in the Sahel (Geddes, 1990). In plague years swarms of the desert locust may attack crops from West Africa to India (Steedman, 1990). Both grasshoppers and locusts frequently require chemical control measures. The campaign against the desert locust outbreak which began in 1985 had cost US$150 million by 1988, with approximately US$70 million of this cost borne by the afflicted countries (Brader, 1988). In 1988 alone, US$100 million was spent on desert locust control (Anon., 1990). Costs for grasshopper control in 1986–87 amounted to US$62 million (Brader, 1988).

The insecticide formerly favoured for desert locust control was dieldrin, which persists for months after application. This was very effective at very low doses per hectare, but is now banned in most countries where control is required because of concern about environmental damage and the hazard to health of humans and livestock. The most recent plague was fought with a variety of less persistent pesticides, of which the most frequently used were fenitrothion and malathion. These are less effective

precisely because they are less persistent, and may give inadequate control (Brader, 1988). As a result of the switch to these somewhat more environmentally acceptable pesticides, control costs have risen tenfold (Brader, 1988). The amounts of pesticide applied have also greatly increased, because several applications may now be necessary instead of only one of dieldrin.

The very high cost of these control operations and the concerns expressed about their effectiveness have led to unease among the donor community, especially in the USA which provides 20% of donor support (Anon., 1990). Brader (1988) noted that the ban on dieldrin should be an incentive to review control strategies and to carry out research on new control methods, including biological control. This approach was reviewed by Prior & Greathead (1989), who discussed the range of biological agents that may attack the desert locust and concluded that the only viable approach was to develop the entomopathogenic Deuteromycotina *Metarhizium* spp. and *Beauveria* spp. as biological insecticides. The reasons for this are reviewed below.

Biology of locusts and their natural enemies

During recession periods between plagues, desert locusts occur as very sparse populations of individuals in the solitary phase, which cause no damage. When conditions for breeding become more favourable, usually as a result of rainfall which provides more areas of soft soil for egg laying, populations increase and the insects switch to the gregarious phase. Hopper bands and adult swarms then form and the latter migrate very large distances by flying, following well-documented wind patterns. Because of the great fecundity of locusts, huge numbers may appear very rapidly when breeding conditions are favourable: a swarm may contain up to 50 million adults/km^2, and in 1988 there were swarms in Sudan covering 150 km^2 (Brader, 1988). A major invasion of swarms may weigh 100,000 tonnes and eat this much green vegetation every day.

If breeding areas were both predictable and accessible, treatment of egg fields or hopper bands would be the preferred approach to control. When dieldrin was used, swaths were laid down in the desert which persisted for many months and effectively controlled hoppers which marched into them. If hoppers are to be treated with non-persistent insecticides it is essential either to treat the whole area or to find and treat every band, but neither of these approaches is feasible. The total areas involved are too great for economic treatment and the environmental damage would also be unacceptable. Surveys for hoppers cannot normally hope to detect more than a small fraction of the bands in a given area, but > 90% must be

killed to give useful control (Brader, 1988). In addition many breeding areas are not accessible due to remoteness or military activity. It is therefore often necessary to adopt a 'firefighting' approach which involves detecting adult swarms and treating them before they arrive at agriculturally important areas.

If adult swarms are the target then a suitable biological agent must have certain properties to be effective against them. Prior & Greathead (1989) noted that although the arthropod predators and parasitoids of locusts had a major role in hastening the end of plagues they were unsuitable for manipulation, and migratory pests such as the desert locust were poor targets for classical biological control. Pathogens showed more promise, because some of these could be manipulated as biological pesticides. This allows the possibility of using conventional application technology, including aerial spraying, which is already highly developed for locust control.

The known pathogens of locusts and grasshoppers include entomopoxviruses of unknown host range and pathogenicity, a single record of a baculovirus in desert locust, several protozoa including the well-studied *Nosema locustae* Canning, mermithid nematodes, several opportunistic, wound-infecting bacteria such as *Serratia marcescens* Bizio and *Pseudomonas aeruginosa* (Schroeter) with a wide host range including mammals, the fungus *Entomophaga grylli* (Fresenius) Batko, sensu lato, and deuteromycete fungi in the genera *Metarhizium, Beauveria, Nomuraea, Paecilomyces* and *Verticillium* (Prior & Greathead, 1989).

To be effective as a biopesticide, the properties of a pathogen must include cheap and easy production, safety and host specificity, application using existing technology, rapid action. Only the entomopathogenic deuteromycetes, especially *Metarhizium* spp. and *Beauveria* spp., fulfil these requirements. They also have the additional advantage of penetration directly through the cuticle, whereas all other agents have to be ingested. These fungi can therefore be used as contact pesticides, and there is no need for them to persist in the harsh desert environment for the target insects to find and eat them before infection can occur. However, it will be necessary to show that they can infect successfully under conditions of high temperature and low relative humidity.

Formulation and application of fungal pathogens for locust control

In 1988/89, IIBC developed a joint proposal with the Biological Control Programme Centre of the International Institute of Tropical Agriculture (IITA) at its station in Cotonou, Republic of Benin, and the Département

de Formation en Protection des Végétaux (DFPV) in Niger for the biological control of locusts and grasshoppers. The core of this proposal was research on the development of formulations of the conidia of entomopathogenic deuteromycetes which would be effective under arid conditions and suitable for application using controlled droplet application (CDA) technology at ultra-low volume (ULV) rates.

The most commonly recorded fungi in this group which attack locusts and grasshoppers are *Metarhizium anisopliae* (Metschnikoff) Sorokin, *M. flavoviride* W. Gams & J. Rozsypal and *Beauveria bassiana* (Balsamo) Vuillemin. Conidia, the natural units of dispersal, and blastospores, which are thin-walled spores produced in submerged culture, are both pathogenic but conidia are more stable. The conidia of *Metarhizium* spp. and *Beauveria* spp. are hydrophobic and difficult to disperse in water, but they are lipophilic and can be suspended easily in oils. Insect cuticles are also hydrophobic, but oils spread readily over the epicuticle which contains a high proportion of waxes. A suspension of conidia in oil therefore adheres readily to the cuticle. The oils may also have the additional advantages of protecting the conidia from desiccation, thus encouraging germination when ambient humidity is low, and spreading them to the intersegmental membranes where penetration may occur most easily. Some evidence for this was obtained by Prior *et al.* (1988), who found that the LD_{50} of conidia of *B. bassiana* for the cocoa weevil pest *Pantorhytes plutus* (Oberthür) (Coleoptera: Curculionidae) was over thirty times lower if the conidia were suspended in a vegetable oil instead of water.

There was no evidence from the work on *P. plutus* that such an effect could be demonstrated at low humidities. However, Marcandier & Khachatourians (1987) showed that when the grasshopper *Melanoplus sanguinipes* (Fabricius) was inoculated with an aqueous suspension of *Beauveria bassiana* conidia, the rate and level of mortality was unaffected by the relative humidity during subsequent incubation, and the LT_{50} was 7–8 days whether the RH was 100%, 33% or 12%.

It thus appears possible to kill grasshoppers by topical application of *B. bassiana* even at very low relative humidity, and to greatly reduce the dose of fungus required by formulating in oils instead of water. Oil formulation would have the additional advantage that conventional CDA equipment could be used, at ULV rates. CDA is a more efficient way to apply pesticides than conventional methods of atomization, but it is essential to formulate in non-volatile diluents to avoid evaporation of the very small spray droplets. Oils are frequently used for this purpose.

CDA techniques are well-adapted to ULV application rates and are essential where the formulation must be applied by air, as is often the case for *Schistocerca gregaria* (Forskål). The technology of ULV application was developed for locust control and the logistical infrastructure already exists. There are also good prospects for controlling a variety of economically

important grasshoppers by ULV spraying. Unlike locusts, it would be feasible to tackle grasshoppers by ground based spraying. The technology for fungus production and application would be in place for grasshopper control at times when locusts are in recession and this would also ensure its availability for locust control in plague years.

Four international donor agencies agreed to contribute to a three year research programme to demonstrate the feasibility of this approach to locust and grasshopper control. These are the Canadian International Development Agency (CIDA), the Netherlands Directorate General for Development Cooperation (DGIS), the UK Overseas Development Administration (ODA) and the USA Agency for International Development (USAID). Work began in October 1989.

Research organization and progress October 1989–April 1991

The programme at IIBC, UK

Are suitable fungal pathogens available?

Surveys are being undertaken in West Africa, Oman and Pakistan for new isolates of suitable fungi, and existing isolates from culture collections are also being tested. An assay technique has been developed using topical application of a standard dose of 75,000 conidia/insect in oil to adult *S. gregaria*. After inoculation the insects are held unfed in plastic boxes at 30°C and mortality is measured over a 7 day period. This method discriminates satisfactorily between the majority of isolates which cause no mortality during this period, and a few which cause mortality beginning on day 4 and rising to >90% by day 6.

All isolates highly virulent to *S. gregaria* which have been tested to date are in the genus *Metarhizium* and have originated from Orthoptera. The isolate adopted as a standard and used in all assays for comparison is *M. flavoviride* IMI 330189 which originated from the grasshopper *Ornithacris cavroisi* (Finot) collected in Niger. Other isolates which are highly virulent to *S. gregaria* are: *M. flavoviride* IMI 324673 ex *Zonocerus elegans* (Thunberg), Tanzania; *M. anisopliae* IMI 168777ii ex *S. gregaria*, Ethiopia; *M. anisopliae* ARSEF 324 ex *Austracris guttulosa* (= *Patanga guttulosa* (Walker)), Australia.

No highly virulent isolates have yet been found originating in non-orthopteran hosts, although some coleopteran isolates showed moderate virulence. No isolates of *Beauveria* spp. have yet been found which are more than weakly virulent to *S. gregaria*, even those which originated from the host itself or other acridids. This may be due to the high temperature (30°C) at which the assays are conducted.

It would appear from the limited evidence to date that *Metarhizium* spp. are more likely than *Beauveria* spp. to be virulent, and isolates from Orthoptera are more likely to be virulent than those from other orders. However, many cases are known of an isolate from one order showing virulence to an insect in another order (Prior, 1990) and it would be premature to draw any conclusions yet on the most favourable sources of useful genotypes. It is clear that highly virulent isolates are available, both from *S. gregaria* and from taxonomically related hosts, even if the latter are geographically isolated from it.

Can infection occur at low humidity and high temperature using suitable diluents?

The relative humidity in the bioassay boxes is approximately 35% and it would therefore appear that these results support the claim by Marcandier & Khachatourians (1987) that infection can occur at low RH following topical application. Mortality also occurs under these conditions following inoculation with an aqueous suspension of conidia, but the mortality peak is delayed by about two days. Comparative LD_{50} determinations for oil and water have not yet been carried out, but it would appear that there is a reduction in LD_{50} with oil formulations similar to that observed by Prior *et al.* (1988).

Are the formulations stable at high temperatures and high incident levels of ultra-violet radiation?

Studies have been carried on the effect of temperature on the survival of conidia of isolate IMI 330189 suspended in various oils. In the most benign oils, such as soyabean oil and mineral kerosene, there was only a 5% loss of viability after 20 weeks at 5° and 15°C, but a similar decline occurred in 2–4 weeks at 25°C and in < 1 week at 35°C. No attempt was made in these experiments to improve stability by the use of additives or by pre-treating the conidia, although several approaches are feasible. It appears that long-term storage will require cooling. Conidia are probably sufficiently stable to allow spraying under desert conditions, but more research is required to improve temperature tolerance.

Ultra-violet radiation in the UVB range is lethal to many fungal spores. A range of compounds is available which absorb UVB and have been used to improve the stability of biological pesticides. Those which are compatible with oils have been investigated in an attempt to improve the survival of conidia of IMI 330189 on exposure to tropical sunlight. Oil suspensions of conidia with added chemical sunscreens have been exposed to the UV component of tropical sunlight using an 'Oriel' brand sunlight simulator.

Oils themselves have a considerable absorbance for UVB but conidial

suspensions in oils without added sun-screens decline to approximately 25% of their initial viability after 60 min exposure and < 1% after 100 min. The addition of a single sun-screen chemical can increase survival to approximately 45% after 60 min, and a combination of two chemicals can increase it to approximately 65%. Care must be taken in selecting combinations of additives, because some combinations have a cancelling effect and the conidial survival is worse than in the unamended suspension.

Can the required lethal dose of spores be delivered to the target?

Under field conditions the biopesticide will impact on the target as very small droplets, probably approximately 75 μm VMD. The lethal dose must therefore be applied in a large number of very small droplets rather than the single large drop used in the laboratory assay. In order to study the deposition of formulated conidia under more realistic conditions, an experimental track sprayer has been built in which the target insects are passed on a conveyor belt at controlled speed through a cloud of spray droplets produced by a conventional CDA rotary atomizer. The technique can also be used to study the effects of varying the droplet size by adjusting the speed of rotation of the atomizer. The experiments are carried out at 30°C and 30–35% RH but these environmental conditions can also be varied. Mortality occurs in 5–8 days depending on dose and droplet size. This suggests that the formulation is suitable for field testing using similar rotary atomizers, which are available commercially as hand-held sprayers.

The programme at IITA, Cotonou

Work at the IITA Biological Control Centre for Africa, Republic of Benin, fits into the joint locust programme as the field site for initial testing of formulations developed in UK, and for basic ecological work on local grasshoppers. The project will also investigate methods of mass production, and initiate a training programme.

Grasshopper ecology

The first six months' data on local grasshopper ecology are summarized in the paper by Paraïso *et al.*, these proceedings. *Zonocerus variegatus* (Linnaeus) is the commonest grasshopper encountered in the humid zone in West Africa. *Z. variegatus* is generally ignored by predators, but is host to a variety of interesting pathogens and parasites, of which *Entomophaga grylli* is the most prevalent. Outbreaks are closely associated with rainfall, but epidemics are very localized, and some mini-climate studies are planned to see if we can predict *E. grylli* outbreaks, and possibly find some way of enhancing them.

Several isolates of *Beauveria bassiana* on *Z. variegatus* have been found, and the best is being developed as a mycopesticide using the same system as for locusts. Despite the controversy over the pest status of *Z. variegatus*, farmers still worry about this insect, and large amounts of insecticide are applied to control it. For these reasons, development of a mycopesticide which could be produced locally and applied by farmers is justified. So long as a pathogen and application system similar to that being developed for use in the Sahel can be shown to be effective, *Z. variegatus* provides us with an accessible model system. Preliminary results have been encouraging.

Of the other grasshoppers in south Benin (Fishpool & Popov, 1984) none are reported as major pests, but local populations can cause damage to a variety of crops. Our interest in them is primarily as a source of pathogens. Project work has focussed on assembling a reference collection of grasshoppers, so that incoming material can be given a positive identification before being dissected to look for pathogens. Some interesting nematodes have been found, and various bacteria and fungi, particularly in *Zonocerus variegatus*.

Field testing of formulations

The main thrust of the work at Cotonou is the field testing of formulations of *Metarhizium flavoviride* developed by IIBC. This work is presented in more detail in the paper by Bateman *et al.* (these proceedings). The most recent field bioassay resulted in 95% mortality in 10 days in adult *Schistocerca gregaria*. However, these insects were infected with *Malamoeba locustae* (King & Taylor) which may have predisposed them to infection. This work is at an early stage of development, and more results will be reported as they emerge.

Production

Fungus spore production is a key element in assessing the potential applicability of any mycopesticide. Fungal spores have been applied at a rate of 2×10^9 spores/ml with 2 to 3 l of formulation/ha (= $4–6 \times 10^{12}$ spores/ha), and we must solve the problems of production if we are to apply sufficiently high doses in the field. With insecticides, it is common to apply 2.5×10^7 LD_{90}s/ha and this is the level we should aim to apply with biologicals too. So far, requirements for arena trials and laboratory bioassay have been met by producing spores in medical flats and various locally available bottles, the most suitable being square-sectioned spirits bottles. Molisch's agar is used as the medium. This method suits oil extraction of the spores very well. Other papers in these proceedings (Roberts; Mendonça) discuss mass production in developing country settings. This

work has not yet been carried out in any African country; it is likely that the Brazilian methods, such as production on rice grains, would provide a better model than methods used in Czechoslovakia or China based on a slightly higher level of technology or experience with fungal fermentation for food production.

Local support

The project has been very favourably received by the plant protection authorities in the Republic of Benin. Issues which we need to face are whether farmers are likely to use mycopesticides, or whether we should think in terms of specialist plant protection teams. In assessing the applicability of proposed methods, it is essential that national programmes are involved early on in the research and development process so that African solutions can be found to African problems.

Training needs which should be addressed at this stage are both graduate specialist training in insect pathology, and training for professional entomologists who are interested in conducting work on insect pathogens. Until we are more confident of our results, we are concentrating on training for research scientists, not for practical implementation.

Environmental monitoring

Environmental monitoring will also form part of our studies. As discussed by Matteson (these proceedings) environmental impact studies are an essential prerequisite to field application. Although pathogens are inherently far more selective than chemical insecticides, and already exist in the environment, the amount of inoculum may be raised above natural levels. We need to know the effect of this on non-target organisms.

The programme at DFPV, Niamey

The DFPV in Niamey, Niger, is predominantly a training organization and provides training for crop protection at a variety of levels. The department also provides a base for exploration and networking activities carried out by the DFPV part of the locust project. DFPV is on the same site as AGRHYMET, and this link provides access to a large body of expertise on acridology.

Exploration and collection

Work started at DFPV in May 1990 with the arrival of the locust control

expert. The first season's field work took place largely in Niger and established a network of collaborators who would be prepared to help collect grasshopper pathogens. This network has subsequently been expanded to all the mainland CILSS countries visited (see paper by Smit, these proceedings); collaborators have been identified in each country. Several notices have been placed in the SAS (Surveillance des Acridiens du Sahel) newsletter produced by PRIFAS. The most recent notice included the offer of cash bonuses for any useful pathogens found; exploration and collection strategies are discussed in greater detail in the paper by Kooyman & Shah (these proceedings).

Subsequent field seasons will be spent travelling throughout the countries of the Sahel and visiting desert locust breeding grounds in Niger and Mali. However, political instability is likely to circumscribe the extent of some of the visits.

Laboratory work

Cultures of *Schistocerca gregaria* and migratory locust, *Locusta migratoria* (Linnaeus), are maintained at DFPV by Mr Tahir Diop, an acridologist of long standing. *Oedaleus senegalensis* (Krauss), *Kraussaria angulifera* (Krauss), *Hieroglyphus daganensis* Krauss and several other pest species have also been brought into culture. By manipulating day-length, it has proved possible to break diapause in *O. senegalensis* enabling continuous reproduction to occur.

Facilities are in place in Niamey for the culture of fungi, and some bioassays of fungi in locusts and grasshoppers have been carried out. Recently, some large cages have been constructed which will allow us to spray locusts with fungal spore preparations under natural conditions and leave them undisturbed after spraying.

Conclusion

The results of this research programme have shown that it is possible to formulate the conidia of *M. flavoviride* in oil diluents suitable for CDA using rotary atomisers and to apply the formulations successfully in the field to kill locusts. Research will now concentrate on improving the formulation, particularly with regard to its stability at high temperatures and its resistance to ultra-violet light. Since isolates of *Metarhizium* and *Beauveria* show considerable specificity it will also be necessary to continue screening for isolates with virulence to other target acridids. The programme has established contacts throughout a vast area of West Africa to provide a source of isolates and, eventually, to participate in the field testing of fungal formulations.

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Section I

Organizations Concerned with Locust and Grasshopper Control, and Country Reports

APHIS; grasshopper integrated pest management in the United States – a co-operative project with emphasis on biological control

G.L. Cunningham

USDA, APHIS, PPQ, Grasshopper Integrated Pest Management Project.

Abstract

The Grasshopper Integrated Pest Management (GHIPM) Project was organized in 1987 in response to record-setting grasshopper infestations that blanketed millions of hectares of US rangeland in the mid-1980s. Initiated as a pilot study, the project is designed to develop and integrate grasshopper control strategies into a total system for use by managers of public and private rangelands. Two areas in Idaho and North Dakota are used as integrated pest management (IPM) demonstration sites. Management studies in these areas include population dynamics, economics, environmental consequences, bait applications, and biological controls. The GHIPM Project is a co-operative effort managed by the Animal and Plant Health Inspection Service in association with other agencies of the US Department of Agriculture and the US Department of Interior, Environmental Protection Agency, State Universities, and rancher associations.

Introduction

Combatting rangeland grasshopper infestations in the western USA is well beyond the abilities of individuals or community organizations. As a result, in 1934 Congress charged the US Department of Agriculture (USDA) with assisting in grasshopper control efforts. USDA's Animal and Plant Health Inspection Service (APHIS) provides direct supervision and leadership for grasshopper management programmes in co-operation with other federal agencies, state departments of agriculture, and private ranchers. APHIS's authority to conduct control programmes is provided by the Incipient Control of Pests Act (1937), the Organic Act of the Department of

Agriculture (1944), the Cooperation With State Agencies in the Administration and Enforcement of Certain Federal Laws Act (1962), and the Food Security Act (1985).

Co-operative control programmes for grasshoppers on rangeland occur every year and were most visible in 1985, when 22 million hectares were heavily infested. In Idaho alone, more than 2.4 million hectares were treated that year. Treatments of this magnitude, usually with aerially applied liquid insecticides in blocks of 4000 hectares or more, raised concern about the effects on nontarget organisms, environmental contamination, and other related problems. The American public and scientists jointly developed considerable interest in integrated pest management (IPM) as a result, and IPM is now the preferred alternative for federal control programmes on rangeland grasshoppers.

GHIPM Project

The Grasshopper Integrated Pest Management (GHIPM) Project was initiated in 1987 as a 5-year pilot programme. Its goal is to develop, evaluate, and implement a grasshopper management scheme that can maintain populations at noneconomic levels and that is cost effective, technically feasible, and environmentally acceptable. Plans are being made to extend the project for three more years, to September 30, 1994, to allow for technology transfer.

The GHIPM Project has its headquarters in Boise, Idaho. Field work is accomplished at two rangeland areas in McKenzie County, North Dakota, and the Shoshone Bureau of Land Management District in Idaho. These rangelands represent the major western ecosystems in which grasshopper outbreaks usually occur. Three distinct study areas are contained in each of the two sites – a no-treatment area, a standard treatment area, and the demonstration area.

No-treatment area

In no-treatment areas, neither APHIS, the GHIPM Project, nor any other co-operator funds or participates in any programme to control grasshoppers. The no-treatment alternative enables the GHIPM Project to study the impact of allowing grasshopper populations to develop without intervention. This study provides a benchmark against which the success rate of other alternatives can be gauged.

Standard treatment area

Within standard treatment areas, APHIS guidelines govern the management of grasshopper infestations. Typically, APHIS implements biological or chemical control methods when grasshopper populations exceed 8 per square yard ($8.7/m^2$). Local factors such as grasshopper life stage and species as well as range condition affect the decision to treat.

Demonstration area

The GHIPM Project tests IPM strategies at selected demonstration sites. Based on grasshopper management needs, these strategies help identify feasible control options.

Research and evaluation of IPM methods in the demonstration area include:

1. formulation of selective grasshopper bran baits
2. development of biological control agents
3. improvement of range management techniques
4. monitoring of environmental effects
5. evaluation of post-treatment effectiveness.

Most of the GHIPM Project's research is done by university co-operators funded with federal tax dollars. Studies fall into one of four categories: forecast, survey, decision making, or options.

Forecast studies are conducted to help predict the time of year when grasshopper outbreaks will occur, the age structure of grasshopper populations during outbreaks, and changes in grasshopper density. Research covers such topics as range management, grasshopper biology, and phenology.

Survey studies are conducted to improve the accuracy of field observations. Research results help establish guides to assist field personnel in identifying nymph and adult grasshoppers. The published field guides portray economically significant grasshopper species, including information on distribution, habitat, and food preferences.

Research on decision making looks at the benefits and costs of grasshopper suppression and the environmental impacts of grasshopper control. Studies cover treatment efficacy, relationships between grasshopper density and forage loss, and the effect of control programmes on threatened and endangered plant and animal species.

Options studies are designed to evaluate current control tactics and to develop new strategies that take into account environmental sensitivity. The project is studying the effectiveness of bran bait formulations for pesticide delivery and possibilities for using reduced active ingredients. Options

studies also involve work on the development and application of biological control.

Biological control emphasis

The GHIPM Project emphasizes research, development, and implementation of biological control for grasshoppers (Anon. 1987, 1990). The 1990s promise to be the decade of the environment with increased public pressure for farmers to lessen their dependence on chemical pesticides. Concern about pesticide residues and ground water contamination by agrichemicals is growing. As a consequence, the use of chemicals is likely to be more restricted in the future. These concerns, coupled with increased federal and state regulations to protect threatened and endangered plant and animal species, push biological control to the forefront as a possible alternative to conventional control techniques.

Unfortunately, the need for research toward the use of biological control agents for grasshoppers has been given lower priority until now because of the availability of inexpensive and effective chemicals. Ranchers and land managers, who expect quick kill with pesticides, will have to readjust their thinking about biological control because it is relatively slow acting. Other barriers, such as economics, mass production, viable shelf life, quality control standards, and governmental regulation, pose significant hurdles to the use of natural controls.

Using combinations of parasites, predators, and pathogens, investigators in the GHIPM Project are addressing biological control on several fronts. The protozoan, *Nosema locustae* Canning is approved for use in Federal co-operative control programmes. Its utility on rangeland continues to be studied. Exotic isolates of the obligate fungal pathogen *Entomophaga grylli* (Fresenius) Batko are undergoing experiments on epizootiology and field release. Entomopox viruses have been isolated from at least eight grasshopper species and studies are ongoing to maximize production efficiency for field release. The microsporidium *Vairimorpha* sp. has been found to cause natural epizootics with mormon cricket, *Anabrus simplex* Haldeman (Orthoptera: Tettigoniidae). Accordingly, studies have been initiated to develop protocols for production of inoculum for field introduction. The fungal pathogen *Beauveria bassiana* (Balsamo) Vuillemin is being investigated to determine its effect on several species of rangeland grasshoppers. Cell culture systems for production of host cell dependent grasshopper pathogens have been initiated along with formulation additive studies for solar-irradiation protection of fungal pathogens. Foreign exploration for grasshopper biological control agents has been focused on Australia and the Soviet Union. Results to date include the collection and quarantine

screening of grasshopper egg parasitoids from the genus *Scelio* (Hymenoptera: Scelionidae). Finally, impact studies of large scale grasshopper control programmes on insect parasites and pathogens continue in the IPM demonstration areas of North Dakota and Idaho.

Co-operative effort

The GHIPM Project is a co-operative effort managed by the USDA's Animal and Plant Health Inspection Service. Other USDA agencies involved include the Agricultural Research Service, Economic Research Service, Forest Service, and Extension Service. Three branches of the US Department of Interior are also co-operating: the Bureau of Land Management, Fish and Wildlife Service, and National Park Service. The US Environmental Protection Agency, 10 state universities, and the McKenzie County North Dakota Grazing Association are also directly involved in the co-operative effort.

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CEC; biological control of locusts with entomopathogens

B. Zelazny

Biologische Bundesanstalt für Land- und Forstwirtschaft, Institut für Biologischen Pflanzenschutz, Darmstadt, Germany.

Abstract

The structure of the CEC locust entomopathogen project is described. The project has only recently started and at present links five European and two African countries. The emphasis is on collection and identification of pathogens, and contact points for three groups of pathogens (bacteria/protozoa, fungi and viruses) are given.

The Commission of European Communities sponsors a project entitled 'Biological control of locusts by entomopathogens'. The project supports research on the use of insect disease agents against locusts and grasshoppers in five European and two African countries. The project has only started recently and little practical work has so far been done. It is scheduled to operate for three years until the end of 1993.

The main objectives of the project are to find diseases for the microbial control of locusts and grasshoppers, to accomplish the necessary isolations, characterizations and testing of such agents, and to develop effective formulations of these pathogens for practical use. These formulations might contain synergistic compounds like chemical insecticides in reduced concentrations or insect growth regulators.

The Institute for Biological Pest Control in Darmstadt, Germany, is the main contractor for the project. The main function of the institute in the CEC project is to co-ordinate activities. It also helps other participants to identify locust diseases and trains staff of African institutes. Its practical research on locust control falls mainly under a national GTZ-sponsored project: 'Biological and integrated locust control'. The institute is part of

the Biologische Bundesanstalt für Land- und Forstwirtschaft (BBA, Federal Biological Research Centre for Agriculture and Forestry). The BBA is presently arranging four associated contracts with European institutions and two subcontracts with African organizations. The following institutes will be participating in the project under these contracts.

The INRA Research Station of Biological Control in La Minière, France, will study mainly the persistence of microbial control agents in the environment, that is how disease agents can survive under the influence of factors such as radiation, temperature or low humidity. The research is led by Dr J. Farques who has extensive experience in this field. Connected to such research will be attempts to improve the persistence of promising agents through suitable methods of formulation. The institute will also search for new pathogens of locusts and grasshoppers and in the past has studied many different types of insect diseases, particularly fungi.

The Laboratory of Comparative Pathology in Saint-Christol-Les-Alès in France is a second INRA institute participating in the project. A group under Prof. M. Bergoin will be mainly involved in the characterization and testing of promising viruses which will be hopefully discovered during the duration of the project. In this connection it should be mentioned that a virus belonging to the group of entomopoxviruses has been recently found in field populations of *Schistocerca gregaria* (Forskål) in the Yemen, but the material has been subsequently lost.

The International Institute of Biological Control in close collaboration with the University of Bath in England is attempting to enhance the infectivity of fungi against locusts through the addition of chemical insecticides (at low concentrations) or insect growth regulators. Dr C. Prior is leading the locust group at IIBC; the work at the University of Bath is headed by Dr A.K. Charnley. If time permits it will also be investigated whether the addition of certain compounds to the food or to baits could facilitate fungal infections through the gut.

A group at the University of Crete under Dr A. Legakis is studying the ecology and control of local species of grasshoppers which are causing crop damage in Greece. The group will mainly attempt to identify diseases suitable for microbial control and to develop methods of producing, formulating and applying these.

The entomological institute of the University of Bologna in Italy has a similar type of programme. The research is conducted by Dr G. Briolini and Dr K. Deseö. The most destructive species of grasshopper is *Calliptamus italicus* (Linnaeus). The ecology of this grasshopper is being studied, especially how populations are regulated by the fungus *Entomophaga grylli* (Fresenius) Batko. The development of microbial control methods will be attempted.

The Desert Locust Control Organization for Eastern Africa (DLCOEA) is starting efforts under the project to find and develop

suitable microbial control agents against the desert locust. Mr Musa of the DLCOEA will be in charge of this work. The research will be done in close collaboration with the participating institutes in Europe. Because the DLCOEA regularly conducts control campaigns in a number of East African countries, it already has a very suitable organization for conducting surveys and field trials.

At the University of Cairo Dr Said Abol-Ela leads a group which will collect locusts and grasshoppers in Egypt and study their diseases. Apart from project contributions this work is strongly supported by ORSTOM. The group and collaborating ORSTOM scientists are mainly interested in viruses. Viruses of locusts and grasshoppers will be isolated, tested and, if found promising, developed as microbial control agents.

Apart from supporting the research work described above, an important function of the project is to encourage collaboration between the participating institutes. Such collaboration was discussed recently at a meeting in Wageningen, Holland. There was a general consensus at the meeting to extend such collaborations also to workers and institutes which are not officially part of the project, if these find such an arrangement mutually beneficial. In fact the meeting was attended by a number of scientists from outside the project. The CEC project is also linked via IIBC to the IIBC/IITA/DFPV Locust and Grasshopper Biological Control Programme and the two projects are co-operating fully in the exchange of pathogens.

Initially a number of areas of collaboration were identified which will increase the overall output of the project. The most important one was mutual assistance in the identification of new diseases. A number of participating institutes are engaged in collecting locusts and grasshoppers, but each worker has only a limited knowledge in recognizing and identifying potentially useful diseases. So that valuable collected material is carefully inspected for all promising groups of insect diseases, the project participants have worked out a system of helping each other in the search for the identification of new diseases. This includes the establishment of three contact points, Saint-Christol-Les-Alès for viruses, IIBC for fungi, and BBA Darmstadt for protozoa and bacteria. Other areas of collaboration would be the exchange of disease agents and locusts for laboratory testing. Some of the disease agents will be used as standards, in order to make results from different laboratories comparable.

Contact points for the identification of insect pathogens:

1. Viruses
   Dr M. Bergoin
   Institut National de la Recherche Agronomique (INRA)
   Station de Recherches de Pathologie Comparée
   F-30380 Saint-Christol-les-Alès
   France
   Tel: (33) 6652 2017

2. Fungi
   Dr C. Prior
   International Institute of Biological Control
   Silwood Park
   Ascot
   Berkshire
   SL5 7TA
   UK
   Tel: (44) 0344 872999

3. Bacteria and protozoa
   Dr B. Zelazny
   Biologische Bundesanstalt für Land- und Forstwirtschaft
   Institute for Biological Pest Control
   Heinrichstrasse 243
   D-6100 Darmstadt
   Germany
   Tel: (49) 6151 44061

CILSS/DFPV; the Permanent Interstate Committee for Drought Control in the Sahel, and the Department of Crop Protection Training, Niger

J.J. Smit

Département de Formation en Protection des Végétaux, Centre CILSS AGRHYMET

Abstract
The Permanent Interstate Committee for Drought Control in the Sahel (CILSS) was set up by nine Sahelian countries following the drought of 1968–73, and agreed in 1974 to improve crop protection services. A joint CILSS/Netherlands mission agreed in 1977 to strengthen training in crop protection with emphasis on integrated methods. The Département de Formation en Protection des Végétaux (DFPV) was set up in 1981 in Kolo, Niger and moved to its present site in Niamey in 1987. The main course lasts two years and leads to a Higher Diploma in Crop Protection. Short language training courses, specialized crop protection courses, and seminars are also held. Dissemination of information is also a very important activity. DFPV is well equipped with modern training equipment.

Following the drought years of 1968–73 six Sahelian countries, later followed by three others, created in 1973 the Permanent Interstate Committee for Drought Control in the Sahel, commonly known by the initials of its French name, Comité Permanent Inter-Etats de Lutte contre la Sécheresse dans le Sahel, CILSS.

This Committee then represented its member states in dealing with their principal donor countries on the subject of support for economic and social development.

Right from the beginning CILSS has given a very high priority to agricultural development, in order to achieve its goal of food self-sufficiency.

The member countries of CILSS are:
Burkina Faso

The Republic of the Cape Verde Islands
The Republic of the Gambia
The Republic of Guinea-Bissau
The Republic of Mali
The Islamic Republic of Mauritania
The Republic of Niger
The Republic of Senegal
The Republic of Chad.

Having experienced serious insect invasions after the drought years, the CILSS countries agreed in 1974:

1. to strengthen their crop protection services and regional institutes for the control of migrating insect pests such as locusts and grasshoppers
2. to develop research and training in crop protection.

After consultations with interested donor countries and international organizations such as FAO and the Club du Sahel, agreements were finally reached for the financial, material and expert support of the following activities:

1. strengthening of national crop protection services
2. development of a system of integrated control methods against pests and diseases of important food crops in the Sahel region
3. development of information services and training facilities in crop protection.

In 1977 a joint mission CILSS/The Netherlands agreed on the kind of training most needed to strengthen national crop protection services in the Sahel and concluded that to protect the environment an integrated control system should be developed, secondly, that there was an urgent need to adapt crop protection training to Sahelian conditions and, thirdly, that there was a most urgent need for mid-level field and laboratory staff in crop protection that could strengthen and improve the efficiency of national crop protection services.

DFPV: a centre for training and information in crop protection

The project for Training in Crop Protection was established in 1981, following a request by the member states of CILSS. It is funded by the Netherlands, following an agreement between that country and the CILSS.

Objective

To contribute to food self-sufficiency by improving the protection of crops and stored products against pests, diseases, and weeds.

The status of DFPV

In 1981, an agreement was signed between the CILSS and the Netherlands to implement a training project for Crop Protection. This project was situated at the Institute for Practical Rural Development (IPDR) in Kolo, Niger.

The urgent need for crop protection expertise, as well as for the various project activities, has been well recognized. Therefore, during the meeting of the Council of Ministers of the CILSS in Dakar, on 25 and 26 January 1986, the member states formulated Resolution No 7/21/CM/86, which conferred permanent status to the Project by creating a Department for Training in Crop Protection, within the AGRHYMET Centre. In January, 1987, the DFPV moved into its new quarters in Niamey.

Two statutory bodies define the DFPV's activities:

1. The Scientific and Pedagogical Committee (CSP)
2. The Management Committee (CG).

The Scientific and Pedagogical Committee is responsible for examining and approving all suggestions of pedagogical and scientific nature, as well as all changes in the orientation of training. The committee is composed mainly of directors of National Crop Protection Services.

The Management Committee, composed of CILSS experts as well as experts from the Netherlands and all other donor countries, approves the budget and accounts of the department as well as the financial consequences of the CSP's decisions.

These two committees meet at least once every year.

Short-term objectives of the Department of Crop Protection Training

The immediate objective of the project is to reinforce the National Crop Protection Services by training medium level personnel working directly in the field.

This is accomplished in part by the dissemination of crop protection information relating to the Sahelian countries but more importantly by the various training activities of the DFPV.

Activities of the DFPV

- A two-year Higher Diploma training course for Crop Protection Technicians (TSPV)
- A four-month French language course for English and Portuguese speaking students from The Gambia, Guinea-Bissau and the Cape Verdian Islands, prior to their two-year training course in Crop Protection mentioned above
- A four-month training course in Crop Protection, intended for the following professional categories:
  - crop protection teachers at agricultural schools
  - senior technicians and agronomists
- Specialization of agricultural technicians and agronomists in their last year of study
- The organization of workshops, covering certain specialized topics in Crop Protection
- The dissemination of information on Crop Protection in the Sahel.

The training course for crop protection technicians

Candidates recruited for the training course for Crop Protection Technicians (TSPV) must fulfill the following requirements:

1. be a national of one of the member states of the CILSS (candidates from non-Sahelian countries may be accepted under special circumstances)
2. possess a baccalaureate certificate, a secondary school certificate or an equivalent certificate in crop protection
3. have preferably two years of professional experience
4. be under 40 years old.

The instruction consists of theoretical courses, as well as practical work in the laboratory and in the field; other appropriate methods are used as needed.

In the first year basic subjects are covered, whereas more specific crop protection topics are treated in the second year. A practical training period and a research project are also part of the TSPV course.

Approximately 25 students are now being trained each year. At the end of the course, after receiving their diplomas, the students return to their duties in their home countries.

Specialization courses

The objective of this education programme is to reinforce crop protection

expertise within the national structures by addressing the following professional categories:

1. crop protection teachers at agricultural schools
2. senior technicians and agronomists working in plant protection and research services.

Training workshops

The objective of these workshops is to broaden the basic understanding of topics of current concern in crop protection; other topics could be covered, as judged necessary by member states.

Crop protection training results

- To date 111 students successfully completed the two-year training course in crop protection and left for their home countries with a Higher Diploma in Crop Protection
- Five students from the Gambia were trained at the Ahmadu Bello University in Zaria, Nigeria
- Twelve students so far followed the French language training course for English and Portuguese-speaking students
- Twenty-five agricultural technicians and higher technicians were given training in crop protection
- Twelve agricultural school teachers were given crop protection training and educational materials were supplied by the project for use in their schools of work
- To date 142 trainees from crop protection services in the Sahel region took part in training workshops organized by the DFPV.

Training courses were organized on the following themes:

1986: Safe and efficient use of pesticides
1987: Phytosanitary aspects of seeds
1988: Rodent pest control
1989: Phytosanitary control
1990: Surveillance of locusts and grasshoppers
1990: Bird pest control
1991: Rodent pest control (April)
1991: Ecology and identification of immature grasshoppers and eggpods (June–August).

Information

The recently created information package is designed to complement the training. Its purpose is to disseminate crop protection information throughout the Sahel.

A database of crop protection documentation has been created at the DFPV, as an integral part of the Sahelian Documentation Network (RESADOC) of the Sahel Institute (INSAH) in Bamako.

Available are AGRIS and CAB ABSTRACTS databases and the DFPV database which will soon contain 4,000 bibliographic references.

Upon request bibliographic searches may be performed free of charge.

Publications

- Descriptive bulletins
- Technical bulletins
- Brochures, technical notes
- Workbooks and lecture notes
- Slides
- Technical documents relating to the workshops
- A newsletter to maintain contact between alumni and other persons connected with the project.

Outside experts are invited to contribute to the publications of the department.

Publications on acridology

The Netherlands Ministry of International Cooperation financially supports the publication of a series of booklets in the French language prepared by CIRAD/PRIFAS, which are being distributed by the DFPV. The series is called *Collection Acridologie Opérationelle* and contains considerable information on acridology for workers in the field.

The following titles are already published:

No 1: Les criquets du Sahel (Grasshoppers of the Sahel)
No 2: La surveillance des criquets du Sahel (The surveillance of Sahelian grasshoppers)
No 3: La lutte chimique contre les criquets du Sahel (Chemical control of grasshoppers in the Sahel)
No 4: *Oedaleus senegalensis*: sauteriau ravageur du Sahel (*Oedaleus senegalensis*: a grasshopper pest of the Sahel)
No 5: Vade-mecum des principaux criquets ravageurs du Sahel
No 6: Le criquet pèlerin au Sahel (The desert locust in the Sahel)

No 7: Les oothèques des criquets du Sahel (Eggpods of sahelian grasshoppers).

In preparation:

No 8: Les ennemis naturels des criquets du Sahel (The natural enemies of grasshoppers in the Sahel).

General equipment of the DFPV

The DFPV has available several types of audiovisual and other equipment (video, slide and transparency projectors) and photographic, printing and photocopying apparatus. The DFPV library is well stocked with publications in the field of crop protection: entomology, ecotoxicology, acridology, ornithology, phytopathology, nematology, virology, phytopharmacy, weed science, rodentology) and related fields of plant and soil science.

Every section of the department has its own systematic collection of insects, fungi, nematodes, weeds, rodents and diapositive slide collections on these important organisms and the damage they cause.

Ample laboratory research facilities for entomology, phytopharmacy, nematology, virology, and ecotoxicology are provided for at the DFPV, as they go hand in hand with practical training of students. An irrigated area of about two hectares is available for out-of-season growing for various crops and three air-conditioned greenhouses built for growing and testing crops are now near completion.

Transport facilities for teaching and research purposes consist of a fleet of three landcruisers, one pick-up, one bus for 30 persons and three smaller cars for the directorate and administration. Two class-rooms for 25 students each are available for regular teaching purposes and there is a spacious conference room for staff meetings, seminars and workshops.

Special equipment in acridology

The DFPV is well equipped for the rearing of grasshoppers in its insectary which is now exclusively used for this purpose. There is also a large mosquito-netted greenhouse which provides facilities for biopesticide tests on grasshoppers.

Rearing of grasshoppers has so far been very successful. *Locusta migratoria migratorioides* (Reiche & Fairmaire), *Schistocerca gregaria* (Forskål) and *Oedaleus senegalensis* (Krauss) are now routinely reared without interruption as we were able to solve problems of reproduction and diapause in the latter species. Rearing of *Kraussaria, Ornithacris* and *Hieroglyphus* will be started in the very near future.

Three pathogenic fungal strains are now being grown at the DFPV, *Metarhizium flavoviride* (W. Gams & J. Rozsypel), *Sorosporella* sp. and *Aspergillus flavus* Link ex Fries. Testing with the pathogenic fungi now available in pure culture will continue on several other species of grasshoppers, notably *O. senegalensis* and *Locusta migratoria* (Linnaeus).

Acridology staff

Two acridologists have been assigned at the DFPV and they co-operate in activities such as grasshopper rearing and surveillance work. They are assisted by four laboratory assistant staff and they have available two drivers for travelling within the Sahel region to visit locust areas. Funding of these activities is by the Dutch Ministry of International Cooperation (DGIS) through the DFPV, which is the home base of the grasshopper project known as the SPC, the French acronym for Surveillance des Pathogènes des Criquets.

CNLA; short report on the control strategy of acridids in Morocco 1987–89

A. Mouhim

Centre National de la Lutte Anti-Acridienne, Morocco.

Abstract

Locust monitoring is carried out by the National Centre for Acridid Control at Ait-Melloul. When action is required the Ministries of Agriculture, Agrarian Reform, Interior and of Information and also the National Defence are also mobilised. Over 2 million ha were treated in 1987–88. The principal strategy is one of destroying adult locusts outside crop zones before they can oviposit.

Introduction

Among the 206 species of locusts and grasshoppers known in Morocco, only two species of locusts cause serious damage to crops. These are the Moroccan locust, *Dociostaurus maroccanus* (Thunberg) and the desert locust, *Schistocerca gregaria* (Forskål). *D. maroccanus* is a native and univoltine species. The control of its gregarious foci has meant that the population has remained at a controllable level. On the other hand, *S. gregaria* is a visiting species which enters Morocco at the end of September to mid-October.

The resurgence of the locust pest problem was encouraged by favourable climatic conditions in the permanent habitat zones of *S. gregaria* and by the paucity of preventative control exercised in the gregarious foci. Thus several uncontrollable swarms formed and engendered an invasion from 1987 to 1989.

In the south and south-east of Morocco there was an unprecedented invasion by massive swarms of locusts coming from Algeria and other

Sahelian countries. During this time, 4,802,805 ha were treated with pesticides.

Organization of acridid control on a national scale

Strategy

The National Centre for Acridid Control (CNLA) at Ait-Melloul does extensive exploration annually in the south of the country, where swarms of *S. gregaria* might infiltrate. If there is much activity in the summer reproduction zones of *S. gregaria* then preparations for preventative control are begun in strategic zones. Usually, this will comprise between three and five aeroplanes and seven to ten 4-wheel drive vehicles with ground spraying equipment and large stocks of insecticide for use over a period of two to three months.

In Morocco, in addition to the people themselves, the Ministries of Agriculture and Agrarian Reform, the Interior and Information, and National Defence are mobilized in the fight against locusts. Co-ordination is undertaken by a Central Command Post (CCP) at Rabat and 12 Regional Command Posts (RCP) in the affected regions.

The CNLA not only contributes to control but ensures technical assistance to every RCP and is concerned with training personnel for locust control. It also ensures that every RCP obtains the pesticides and equipment which it needs.

More than 3,600 people from different ministries took part in control operations; 28% were technicians or qualified research scientists. Sprayers used were atomizers, hydraulic pressure sprayers, and exhaust nozzle sprayers mounted on vehicles. 57 aeroplanes were used of 13 different types, depending on the topography of the area and with a capacity of 200–12,000 l. In addition, there is a well established radio network with 108 radio posts, 66 mobile and 42 fixed.

The pesticides used were selected on the basis of toxicological data (rapid action against the locust, but less toxic to man, fauna and flora), the types of sprayers which were available and storage conditions. 5,981,146 l were sprayed at low dosages: 0.8–1 l/ha by air and 2–5 l/ha on the ground (Table 1).

In 1987–88, 2,813,182 ha were treated, 1,201,245 against larvae and 1,611,937 against adults. In 1988–89, acridid populations were exclusively swarms of immature locusts. 1,989,622 ha were treated, thus, the total in these two seasons was 4,802,805 ha.

During the two years 0.85 billion francs were spent on fighting acridids, 54% of which was donated from abroad, with a cost of US$12/ha.

Table 1. Volumes of insecticides and carrier used in Morocco during locust campaigns 1987/88 and 1988/89.

Product	Dose (g a.i./ha)	Amounts used (litres) 1987–88	1988–89	Total (litres)
Dichlorvos (DDVP 96%)	200	88,702	401,251	489,953
Fenitrothion (1000 ULV)	350	412,790	4,400	417,190
Fenitrothion 500 ULV	350	120,600	48,400	169,000
Malathion 96% ULV	700	781,526	661,327	1,442,853
Carbaryl 4% oil	480	42,784	–	42,784
Diazinon 90% ULV	900	8,200	–	8,200
Deltamethrin 12.5% ULV	12.5	0	56,700	56,700
Deltamethrin 25% EC	12.5	0	22,325	22,325
Lambdacyhalothrin 4% ULV	20	8,900	5,825	14,725
Organic diluent for ULV formulations (HAN)				
Total		1,900,000	1,417,416	3,317,416
		3,363,502	2,617,644	5,981,146

The acridid problem is not just a national one, but regional throughout the Sahel and even international. In this context a conference was held in Fes in 1988 with the idea of creating an international acridid control force. The aims of this body are to reinforce exploration and control of the deser locust and to ensure a better evaluation of the acridid situation. There are three units in Mauritania, Mali and Tchad and two others in Sudan and on the Red Sea.

Conclusion

The invasions of locusts between 1987–89 were the largest this century. The success of control measures prevented enormous damage. The efficiency of the interventions are based on the following:

1. the adoption of a new concept in acridid control, that of destroying adults outside crop zones before oviposition
2. the use of qualified and experienced personnel
3. the improvement of spraying techniques
4. the selection of more appropriate methods of control according to the size of swarm and the kind of terrain
5. the increased use of large trucks and aeroplanes.

Morocco has become one of the pilot countries in the organization of acridid control, thanks to the enriching experience undergone during the last few years.

DLCOEA; pest control: the begging questions

R.A. Kitenda

Desert Locust Control Organization for Eastern Africa.

Abstract
The possible ways in which schemes for the biological control of locusts and grasshoppers might fit in with existing modes of operation are discussed. Particular attention is paid to the problems experienced by locust control organizations in recent years with a run-down in resources.

Biological control of locusts and grasshoppers is a subject I am not at all familiar with. However, looking at the approach being made from an educated and experienced view point, I am of the opinion that a great step indeed is being made.

It is a great step considered in relation to past and present measures and means of locust control in my area of specialization which is aerial control.

The biological control programme when being developed is likely to have some teething problems and perhaps when fully evolved would have its limitations just like any other. It is however, a very welcome element in an integrated pest control approach in general. We must endeavour to reduce poliutants.

Aerial control of locusts and grasshoppers (area of specialization)

Locusts have existed for as long as man can remember. Various ways and means have been employed in every effort to try and contain the scourge

since the beginning of history; some very crude and others refined as time went by. All these measures have succeeded to some extent in containing the threat. The degree of success by the means employed has, however, depended to a large extent on the size of infestation and the experience and expertise of personnel employed. This state of affairs is as true today as it was in yester years and it will remain tomorrow, whatever the methods.

Constraints

There are various bottlenecks which have and still continue to afflict effective locust and grasshopper control:

1. finance
2. trained manpower
3. effective monitoring
4. research.

The financing of control organizations remains a very big problem and in many cases a burden to participating states. This situation is not helped in any way when conservation of resources is undertaken by incompetent personnel.

The future can only be improved if the burden is carried by the international community and management handed to competent heads with sufficient expertise. The donor community must reconsider assistance strategy.

Trained manpower in the area of locust control is almost extinct. The reasons are manifold, poor remuneration, difficult working conditions, poor recruitment decision making, and again lack of funds and facilities.

Unnecessary amounts of pollutants are still being dispersed in the atmosphere because of untrained manpower incapable of equipment calibration and adjustment.

Without ways and means to effectively monitor areas of outbreak and breeding, it is impossible to cope with the demand of the job regardless of the method employed. The task of monitoring has unfortunately become increasingly difficult, given that many key geographical areas are also zones of conflict. We must therefore seek alternative ways of gaining access to breeding territory using available technology to reduce outbreaks. The question remains how to adapt and utilize such technology.

Research in locust and grasshopper control has for a long time now been dormant. The advent of biological control research is therefore very welcome.

Where there is research being conducted, it is incomplete, in that it ends in the laboratory without being extended to real field trials.

Research does not have continuity and therefore leaves unfilled gaps. In many instances researchers leave their jobs taking the records of their work with them.

The list of deficiencies in locust research is long but one of vital importance is the failure to establish the economics involved as in the final analysis everything depends upon money.

This means that the next person on the job has to start afresh or on very scanty information. Since locust outbreaks occur after lapses of time, some many years apart, the would-be researcher may never have fulfilment of knowledge gained in the laboratory under actual field conditions. We know the history of control measures, we have the present in hand, but what does the future hold in store when we consider the experience gained to-date! The future survival of locust and grasshopper control lies in a co-ordinated international effort through a recognized body with a specialized group of experts.

The biological control approach is welcome and must be pursued relentlessly as part of an integrated effort.

The programme must avoid the present pitfalls in existing programmes; namely, allowing a run-down of trained manpower, gaps and deficiency in research, employing incompetent or disinterested staff and over-extending resources; high-tech application must be seriously enhanced to help in reducing the pressure on limited resources; we know how far history has brought us today. We must therefore build a more certain future based on knowledge so far gained and the desire to reduce the threat of locusts and grasshoppers to our food supply.

GTZ; the integrated biological control of locusts and grasshoppers – a GTZ research project

S. Krall & O.M. Nasseh
GTZ-Projekt.

Abstract
The aim of the integrated biological control project of the GTZ is to develop more acceptable alternative non-chemical methods of controlling locusts and grasshoppers. The existing control measures still rely heavily on chemical pesticides which are the only method available at the present time for cases of severe infestation. The most effective pesticide, dieldrin, is banned in most countries. The substitutes are less effective, more expensive and require multiple applications. It is therefore essential to search for more acceptable, non-chemical alternatives. Preliminary tests have shown that one such alternative could be the use of pathogens and insect growth regulators. The final goal of the project is to localize and attack the problem at the earliest possible stage of development of locust upsurge. This will comprise identifying the problem in the recession areas of the desert locust (*Schistocerca gregaria* (Forskål)) and then treating the insects. In this way the impact on the environment is minimized, the required treatment area is limited, the costs of control are reduced and the potential for crop and grassland damage is negligible. An additional biological, non-chemical alternative method would support this strategy. The programme of the project has nine principal objectives:

- mapping the locust habitat by satellite
- research into gregarization pheromone, to prevent swarm formation
- locust control with insect growth regulators, especially synthetic juvenile hormone analogs
- biological control of the locust with entomopathogenic micro-organisms
- pest management with the help of pyrrolizidine alkaloids
- seed components from *Melia volkensii* and their use for locust control
- field trials with non-chemical alternatives
- research on environmental aspects of the new compounds

loss assessment and economic studies relating to the effects of locust and grasshopper attack/damage.

Introduction

From 1985–88, after an interval of almost twenty years, outbreaks of the desert locust (*Schistocerca gregaria* (Forskål)) occurred in Africa and the Near East. Climatically favourable conditions in conjunction with the neglect of the warning services and the inaccessibility of some areas due to armed conflict contributed to this mass reproduction. The countries affected were largely unprepared and poorly equipped to cope with the plague. Moreover both donors and recipient countries discovered that since the last plague, hardly any research had been carried out into developing better and more environmentally acceptable control methods. An additional handicap was represented by the banning of the insecticide dieldrin. The advantage of this insecticide, which had been used in all previous plagues, was that, due to its persistence it needed to be applied in the breeding grounds only in widely spaced bands, at large intervals. On crossing these bands the larvae became contaminated and died. When dieldrin was banned the effects of this special method of locust control in Africa had not been analysed sufficiently. Accordingly no alternative agent or methods had been developed to replace dieldrin and in the case of the recent outbreaks, this resulted in mainly short-life insecticides being applied. This in turn resulted in much larger areas having to be treated, and in multiple applications, with all the accompanying negative effects on the environment. On the basis of this unsatisfactory situation a discussion was instigated on the promotion of research into new, biological and integrated control methods. Programmes along these lines were elaborated at international level. The Deutsche Gesellschaft für Technische Zusammenarbeit (GTZ) submitted a proposal to the Federal Ministry for Economic Cooperation (BMZ) who made almost 6 million DM available for research into integrated biological control of locusts and grasshoppers. Under the leadership of the GTZ and German research institutions in co-operation with international institutes and organizations as well as technical co-operation projects, research programmes were begun. The first phase of the project will be a three year term which will deal chiefly with practice-oriented research approaches. These approaches are outlined briefly in the following pages. It is intended that the results will be published at appropriate intervals. Field trials in the Sudan, on the Cape Verde Islands and in the Republic of Niger have already produced interim results.

Approaches for research into alternative methods of locust control

Early and remote sensing

Control measures for locust pests can be made environmentally more acceptable by identifying potential epidemics at their beginning and initiating control measures in good time. This is currently effected primarily by surveys and information from the local population, in particular from nomads. This system is efficient but it cannot always be implemented globally and will now be supported by the most modern methods of satellite photography. By evaluating satellite photos (Landsat), maps will be drawn up on a model basis for the Red Sea coast of Sudan, identifying areas which are potentially suitable for mass reproduction of locusts. Such satellite maps, in combination with normal maps, could be of great assistance to prospectors and pilots in the performance of their tasks in future. The present state of technology does not permit a direct identification of locusts by satellite, even when they appear in swarms. The programme outlined here will be implemented under the leadership of Professor Voss at the Institute for Geography of the Technical University Berlin.

Prevention of swarm formation by pheromones

Schistocerca gregaria occurs in a solitary and in a swarming (gregarious) form. In the solitary phase the locusts live in isolation, inconspicuously and as a useful element of a labile ecosystem in areas known as recession areas. Sustained favourable conditions of life lead to explosive mass reproduction. This is accompanied by a gradual change from the solitary to the swarm-forming (gregarious) phase. Adult *S. gregaria* occurring in vast aggregations are able to fly very long distances. They can thus reach areas of agricultural use and cause devastating damage. It appears that the aggregation may be induced by pheromones which influence the behaviour, the appearance, and many of the physiological properties of the locusts. So far none of these potential phase pheromones has been identified, nor is anything known about where they are formed, or their chemical nature. Under the leadership of Professor Ferenz, attempts will be made in the Department of Biology at the University of Oldenburg to identify, isolate and analyse the chemistry of the gregarization pheromone(s). Once the pheromone is synthesized it will be possible to examine the formation and the mode of action of the substance in the locusts. From the resulting knowledge of such a gregarization pheromone and the biological foundations of gregarization, possibilities for targeted intervention in the gregarization process will be developed. However, it is not yet possible to define the exact details of this intervention scheme.

Locust control with juvenile hormone analogs

Juvenile hormones are formed in the insect. They are inter alia, antagonists of the metamorphosis hormone, ecdysone. They prevent metamorphosis to the adult insect; in the case of *S. gregaria*, for instance, there are between five or six larval stages. It is only at the final moulting that the juvenile hormone titre drops so low that an imaginal moulting to the complete insect stage takes place. A synthetic increase in the juvenile hormone titre by additional administration brings about disturbances in the moulting process. The insects die since they are unable to free themselves from the old larval skin. Furthermore, juvenile hormones play an important role in the phase change of *S. gregaria*, affecting the mating behaviour of the male, the egg maturation in the female and possibly the accumulation of lipids (energy reservoirs for the long migration flights). Juvenile hormones have been biochemically analysed so that in the meantime it has been possible to synthesize more than 2,000 analogs. Under the leadership of Professor Dorn of the Institute of Zoology at the University of Mainz, juvenile hormone analogs from as many different chemical groups as possible will be screened for effective interference with the above processes. Tests to investigate the active mechanisms will also be conducted. This concerns both the role of the endogenous juvenile hormone in disturbing physiological processes, and the effects achieved by the analogs. Alongside the juvenile hormones, the so-called anti-juvenile hormones which have not yet been the subject of much research will be examined.

Locust control with entomopathogenic microorganisms

Locusts are attacked by viruses, bacteria, fungi and protozoa. It is the task of the Institute for Biological Pest Control at the Federal Biological Institute for Agriculture and Forestry in Darmstadt (Biologische Bundesanstalt für Land- und Forstwirtschaft) to conduct surveys of the occurrence of microbial antagonists of various types of locust and grasshopper, of their isolation, characterization and behaviour in response to environmental factors, and to test their effectiveness under laboratory conditions (Zelazny, these proceedings). To this end, specimens of dead or suspected diseased locusts and soil samples from various regions of Africa are collected and screened. Both new and previously recognized pathogens are tested in the laboratory for their effectiveness and their environmental behaviour. The aim is to elaborate formulations which can then be used in the field.

Pest management on the basis of pharmacophagous reactions to pyrrolizidine alkaloids

Pyrrolizidine alkaloids are secondary plant substances which occur in many plant species and serve primarily as protection against being eaten. Professor Boppré of the Institute of Forestry Zoology at the University of Freiburg is heading research into the relations between a specific locust species (*Zonocerus variegatus* (Linnaeus)) and one of its host plants which contains pyrrolizidine alkaloids (*Chromolaena odorata*). The alkaloids are taken up by the locusts and exercise the same function as in the plants – protection against being eaten. They do not serve nutritional purposes. For this reason such behaviour is termed pharmacophagy. The plants exert a strong attraction on the insects. Whether the alkaloids have additional influence on *Z. variegatus* is a subject for research. It is known that certain butterflies use pyrrolizidine alkaloids as precursors in the synthesis of sexual pheromones. The goal of the research is to use the alkaloids for the production of bait-like substances, and thus lure the locusts so as to tackle them selectively in small areas. Field trials are being conducted in the Republic of Benin (West Africa). The extent to which this insect-plant relation exists in *S. gregaria* also is to be investigated in preliminary laboratory tests.

Plant components as locust control agents

As in *Azadirachta indica* (neem), compounds suitable for insect control also occur in *Melia volkensii*. Under the leadership of Professor Rembold, the Max-Planck Institute in Martinsried will investigate this component. Seeds of the plant are to be collected and treated in co-operation with an international institute in Kenya. The active agents will be isolated and analysed. Laboratory tests will be conducted with crude extracts in order to test the development-inhibiting impact on locusts. By clarifying the structure of the active agents it may be possible to provide initiatives for the synthesis of new control agents.

Test of new products under practical conditions

There are already new kinds of product on the market or at the checking stage, which might represent promising alternatives to the synthetic insecticides employed so far. These include in particular the development of moulting inhibitors. They act in a manner similar to that of the juvenile hormone analogs already described. Pathogenic micro-organisms which have been formulated as control preparations also exist. These and the other preparations derived from the previously described research approaches must be tested under field conditions. Only in this way will it

be possible to assess their potential for subsequent large-scale application. Testing of these agents will be conducted under the leadership of the GTZ in various African countries. The microsporidian *Nosema locustae* Canning (as bait formulation) has so far been tested on the Cape Verde Islands against *Oedaleus senegalensis* (Krauss) and *Diabolocatantops axillaris* (Thunberg). In 1991 series of trials were conducted in Niger. In field cage trials, beside the pathogenic effects of *Beauveria bassiana* (Balsamo) Vuillemin and *Nosema locustae*, the impact of neem and teflubenzuron were tested on *S. gregaria*.

Parallel ecotoxicological tests

Since the new kinds of products are not necessarily environmentally benign, even though they possess a lower degree of toxicity for warm-blooded animals than conventional insecticides, a continuous ecotoxological back-up programme will be implemented. Professor Müller from the Institute for Biogeography of the University of the Saarland heads a team which is backing up all practical trials with tests for possible side-effects of the agents employed. This has already been implemented for the Sudan, the Cape Verde Islands and Niger. In addition, preliminary tests with new active agents will be conducted in the laboratory.

Studies on economic aspects and loss assessment

Our present knowledge concerning the potential and actual damage caused by locusts to food crops and pasture plants is extremely meagre. Under the direct leadership of the GTZ losses on millet will be investigated and economic calculation models will be set up. Model field tests are being conducted in Niger in co-operation with the GTZ plant protection project. Papers on losses due to grasshopper and locust damage and on loss assessment on millet in Niger are available.

Prospects

The initial term of the project is three years. For this reason approaches have been selected which may reasonably be expected to produce results within this period. These approaches include testing the latest generation of preparations, research on pathogenic micro-organisms, or production of bait based on pyrrolizidine alkaloids. Other components, such as research into pheromones, must be considered as more long-term programmes. Should this first phase of the project be successful and further research appear necessary and promising, an extension could follow.

IAPSC/OAU; communiqué from the Inter-African Scientific Secretariat of the OAU, with comments on the application of the Inter-African Plant Protection Convention

N. Nkouka

Organization of African Unity.

Abstract
The Organization of African Unity approved a Phytosanitary Convention for Africa in 1967 and resolved to make member states apply it. The implementation of quarantine precautions varies among states, and breaches of quarantine which have occurred in other parts of the world serve as examples for the need for vigilance in maintaining quarantine standards at the national level. However, the convention allows member states to import small quantities of prohibited material for research purposes provided they observe precautions recommended by the OAU.

Your Excellency the Minister, Representatives of International Organizations, Honourable Delegates, Ladies and Gentlemen.

In the name of His Excellency, Dr Salim Ahmed Salim, Secretary General of the OAU, I should like to thank IITA for associating itself with our organization in participating in this workshop on the biological control of locusts and grasshoppers. In fact, the felicitous convergence of IITA projects dealing with plant protection in Africa and of the OAU's Inter-african Phytosanitary Council, demonstrates, if indeed it were necessary, the concerns of our countries.

The fact that we are here today to work in Cotonou in the Republic of Benin, proves again how officials of State and the people of Benin have remained true to the great African qualities of hospitality, availability, dialogue. We thank them gratefully for this.

We have taken this opportunity of reminding you in this message from the OAU, which I have the honour to bring to this workshop, and as a base-line from which to work, of the guide-lines given in Resolution 119

(IX) of the OAU Council of Ministers held at Kinshasa in September 1967. This adopted resolution states, and I quote:

'The Council of Ministers of the Organisation of African Unity, assembled in its ninth ordinary session at Kinshasa from the 4th to 10th September 1967.
— Having examined the proposal to establish a continent-wide phytosanitary convention for Africa, to protect plant health;
— noting that the scientific council for Africa, which held its second session at Addis-Abeba, Ethiopia from 8 to 12 April 1967, recommended that this convention should be accepted;
— noting, moreover, that the OAU charter advocates the coordination, harmonisation and intensification of the activities in such areas as these; we are convinced that if properly applied, this convention would allow us better to control the pests and plant diseases which rage in Africa and in this way to increase production and economic yields.

It is decided:

a. To approve the Phytosanitary Convention for Africa:
b. To make the member states of the OAU apply it.'

These provisions give quite precise directives to member states on taking proper quarantine precautions control and inspection measures and in general any measures judged necessary by the OAU, concerning organisms living off plants, parts of plants, seeds, land, manure or packing materials, including containers and all articles which the OAU declares dangerous to agriculture throughout Africa.

No provision in the Articles of the Convention prevents any member state from importing small quantities of plant or other prohibited material, whilst observing precautions recommended by the OAU, in order to do basic or applied research. It is understood however, that such imports of prohibited material will only be made if absolutely necessary. The specific member states will inform the OAU of all such imports which are normally prohibited and the OAU in turn will inform other member States.

Constraints to the application of the Inter-African Plant Protection Convention

Despite the adoption by Ministers of the OAU of the Inter-African Phytosanitary Convention, it must be stated that its application by the member states and partners does not seem satisfactory. We cannot doubt the political will of the authorities since the legal framework exists.

During this workshop on biological control of locusts and grasshoppers there are at least two issues which we should address, since there may well

be imports of live organisms to the continent for this kind of work. These two issues are at different levels:

1. the existence of national legal documentation concerning plant protection regulations
2. the implementation of these laws on plant protection legislation in our countries.

It should be noted that the situation varies considerably from country to country. In fact, Ivory Coast, Senegal and Cameroun already have quite solid legal barriers concerning plant protection.

It should be remembered that all member states of the OAU have ratified the Inter-African Phytosanitary Convention. Another question which could be asked, is why the adoption of national plant protection regulations runs into difficulties. Here, I ask for action from the Directors of National Plant Protection Services who are, in point of fact, technical advisers in this domain to their governments.

Scientific and popular knowledge seem, in the final analysis, to be two of the determining factors in decision making and in the perfection of the responses necessary when faced with the problems of plant protection regulation.

These issues are in everyone's interest. We scientists know what happened, in Europe, for example, concerning the pests of potato and grape vines. African countries can and must take these catastrophes as warnings.

Prevention depends on what responses are possible. These responses can be translated into documentation, attitudes, particularly when people travel, and in migrations. People should be aware that taking a plant or an insect in a handbag across borders, can create danger for a whole country.

Economic crises and agricultural production particularly require rapid solutions and in these conditions practising research scientists will, from time to time, have to convince our decision makers on the necessity for introducing such and such an organism in order to fight a specific pest; thus, in the name of the Inter-African Phytosanitary Council of the OAU, for whom you are the prime movers chosen for and privileged by your active participation in the creation of coordinated phytosanitary regulation in each country, take every care in order to avoid wrong decisions being made as the result of contradictory interpretations.

Today, we are engaged in biological control of locusts and grasshoppers in the same way as yesterday we fought against *Rastrococcus invadens* Williams and *Phenacoccus manihoti* Matile-Ferrero (Homoptera; Pseudococcidae). It seems that here we have two examples which may help us better to ascertain possible constraints for future projects.

In passing, I should like to mention that a consultative scientific report initiated by the Phytosanitary Council on *Zonocerus variegatus* (Linnaeus)

is available at our offices in Yaoundé, Cameroun and we would like to make effective use of this study through a network of researchers in collaboration with IITA and other institutions.

Whilst reminding you of the legal framework for introducing live organisms to our continent, we have an opportunity for the OAU to lend its support to federal biological control programmes which are carried out by various international organizations.

Perhaps you already know that the OAU wishes in the near future, to redefine itself as an Economic Commission for Africa and the agro-food dimension will be seriously considered in all its aspects, including that of research.

Your contribution to the work in progress will be faithfully relayed to the Secretariat General of the OAU.

I wish you every success for this workshop and thank you for your kind attention.

ICIPE; development of a biocontrol strategy for the management of the desert locust, *Schistocerca gregaria*

S.K. Raina

International Centre of Insect Physiology and Ecology.

Abstract

Intervention for prevention of locust swarms may be contemplated either by disrupting gregarisation or manipulating solitarious populations to keep numbers low. The former could be achieved by manipulating pheromones and the latter by the augmentation of indigenous natural enemies. Protozoan and viral pathogens may have potential for use in arid conditions because the protein coats render them resistant to extremes of temperature. They also show host specificity and transovarial transmission. ICIPE has initiated a programme to select and evaluate these agents for introduction into pregregarious populations, with emphasis initially on the protozoan *Malamoeba locustae* and entomopoxvirus.

The desert locust, *Schistocerca gregaria* (Forskål), has a vast invasion area of around 29 million km^2 in which farming communities from 57 countries in the continents of Africa and Asia are affected. The devastating outbreaks of locusts in Africa which have plagued the mid-1980s, reflect a long-lasting problem with chemical control. The current method of control is mainly based on application of toxic chemicals which are detrimental to the flora and fauna of the treated areas and can result in the development of new pests because of the disruption of the natural enemy complex. Moreover, the present control strategies become operative only when locusts reach outbreak status. In ICIPE's view, prevention of locust outbreaks would be a more attractive management strategy than attempts to control populations that have already reached swarm levels. Conceptually, at least two levels of intervention, which may pre-empt or prevent the formation of swarms are possible:

1. the first involves the disruption of the process of gregarization
2. the second involves the manipulation of the population equilibrium of the solitary locust, to levels less likely to develop into swarms.

Successful research and development leading to viable interventions at these levels through the induction of chronic diseases, could virtually contain the locust menace and minimize the present reliance on large-scale use of environmentally detrimental insecticides.

Some research groups working on biocontrol of locusts have been engaged in developing biocides for direct hit on desert locust swarms. This is basically different from our approach at ICIPE, which focuses on colonization of selected pathogens, augmentation of indigenous inoculum to induce an epizootic, and conservation of inoculum following enzootics or epizootics within pre-gregarious locust populations.

Due to the severe conditions prevailing in the desert locust recession and breeding zones, candidate pathogens which require high humidity, such as fungi, and those which cannot perpetuate in the desert environment (bacteria and nematodes) are considered unsuitable components of a sustainable locust management package. However, some protozoans and insect viruses, if appropriately formulated, could tolerate the extremes of temperature and humidity because of their protein coverings. Both groups can perpetuate through transovarial transmission and are known to be specific to their target hosts (Henry, 1971; Summers *et al.*, 1975; Menapace *et al.*, 1978; Kurstak *et al.*, 1978; Canning *et al.*, 1985; Henry, 1985; Raina *et al.*, 1987, 1990).

In view of the envisaged potential of microbial control, ICIPE has initiated a biocontrol research programme, based on the above concept, which is to be executed in two phases:

1. to evaluate, isolate and characterize virulent protozoan and entomopoxviruses likely to be associated with desert locusts in their recession and breeding areas

2. (a) to develop methods for colonization and augmentation of the effective micro-organisms in selected pregregarious populations
   (b) to devise production and formulation technology for field release.

Investigations in phase 1 have started using initially the known protozoan candidate (*Malamoeba locustae* (King & Taylor)). Other protozoans and viruses are also being considered. The biochemical characterization, pathogenicity evaluation through bioassay techniques, virulence improvement by selection procedures and extensive biosafety tests, would make it possible to establish the potential of these protozoans and viruses as biocontrol agents for sustainable management of the pregregarious population of the desert locust. A thorough study of the population dynamics of pregregarious desert locust in recession areas

would help develop the proper methodology for dispersal of these micro-organisms in the field after proper formulations. This is expected to be part of a comprehensive strategy, integrating biocontrol agents with selected semiochemicals (pheromones and kairomones).

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IRLCOCSA; perspectives and prospects of biological control of the red locust, *Nomadacris septemfasciata*

J.W. Bahana & E.K. Byaruhanga

International Red Locust Control Organisation for Central and South Africa.

Abstract

The increasing awareness of environmental safety in recent years has brought to the forefront the dangers posed by chemical pollution from insect pest control. Although advances have been made in the search for alternative strategies to chemical pest control, the successes of such methods have been limited to a few insect pests.

Locusts pose formidable problems and their control has so far been possible only through intensive chemical spraying. Hitherto, other methods have not had any significant impact on prevention of locust outbreaks. On the other hand, the success of chemical control appears to have masked the development of alternative control methods.

The red locust, *Nomadacris septemfasciata* (Serville), is no exception to the locust story, but this locust has peculiarities in its ecosystem that allow alternative control strategies. This paper reviews areas in which such strategies could be developed and proposes that the red locust can be a model in the development of biological control for locusts in general.

Introduction

The red locust, *Nomadacris septemfasciata* (Serville), has been responsible for four major plagues in Africa south of the Sahara in the past 150 years but little is known of the first two. Records show that there were plagues about the periods 1847 to 1854, 1892 to 1910, 1913 to 1919, and 1930 to 1944. However, most of the information on red locust plague dynamics and progression is available only for the 1930 to 1944 plague when extensive

studies were carried out to try and find a solution to the locust menace.

Evidence from the studies revealed that *N. septemfasciata* plagues originated from definite source areas where simultaneous and widespread breeding was initiated. Consequently, there were swarm escapes and further breeding both inside and outside the source areas. After a period which varied with each plague, there was collapse of locust populations. Later, breeding was limited to the source areas, and at very low levels.

Control methods employed during the first three plagues were rudimentary and ineffective. Although chemical pesticides, particularly arsenic compounds, were applied in an attempt to control locust upsurges, their use was not widespread but was restricted to the source areas. It can hardly be claimed, therefore, that pesticides were effective in ending the red locust plague. Moreover, the more effective acridicides that were introduced, such as dieldrin, came on the scene only much later, after the plague had dissipated. So, what triggers population upsurges that initiate plagues, and what is responsible for the population collapse? What role did ecological factors play in bringing about the collapse of *N. septemfasicata* populations, four times in the last 150 years? Was there a significant role by biocontrol agents in these wild fluctuations?

The ecology of *N. septemfasciata* and its outbreak areas

During the last plague of 1930–44, swarms of the red locust covered virtually all the areas south of the equator, and even as far north as Sudan and Somalia (Steadman, 1990). Studies, in part triggered by the effects of the recurring plagues, were initiated in the 1930s to try and find the origin of plagues, with a view to stopping them at source. The search for outbreak areas was based on the theory of the great acridian scientist Sir Boris Uvarov, who postulated that plagues were initiated from a few special ecosystems, later to be referred to as 'outbreak areas'.

Field investigations and subsequent analysis of reports of locust occurrence led to the conclusion that swarms which had apparently started the plague had initially formed in the grass plains of Mweru-wa-Ntipa in northern Zambia. It appeared that another source area was the Rukwa Valley in Tanzania, but there is no concrete evidence to support this (Symmons, 1978). Areas which were later found either to have produced migrating swarms, or to be capable of producing one if not controlled, were designated recognized outbreak areas (Symmons, 1978). Since 1944, these outbreak areas (Fig. 1) have at one time or another produced swarming populations (Table 1).

The outbreak areas of *N. septemfasciata* have a number of common ecological features which have been described by Gunn (1955, 1956,

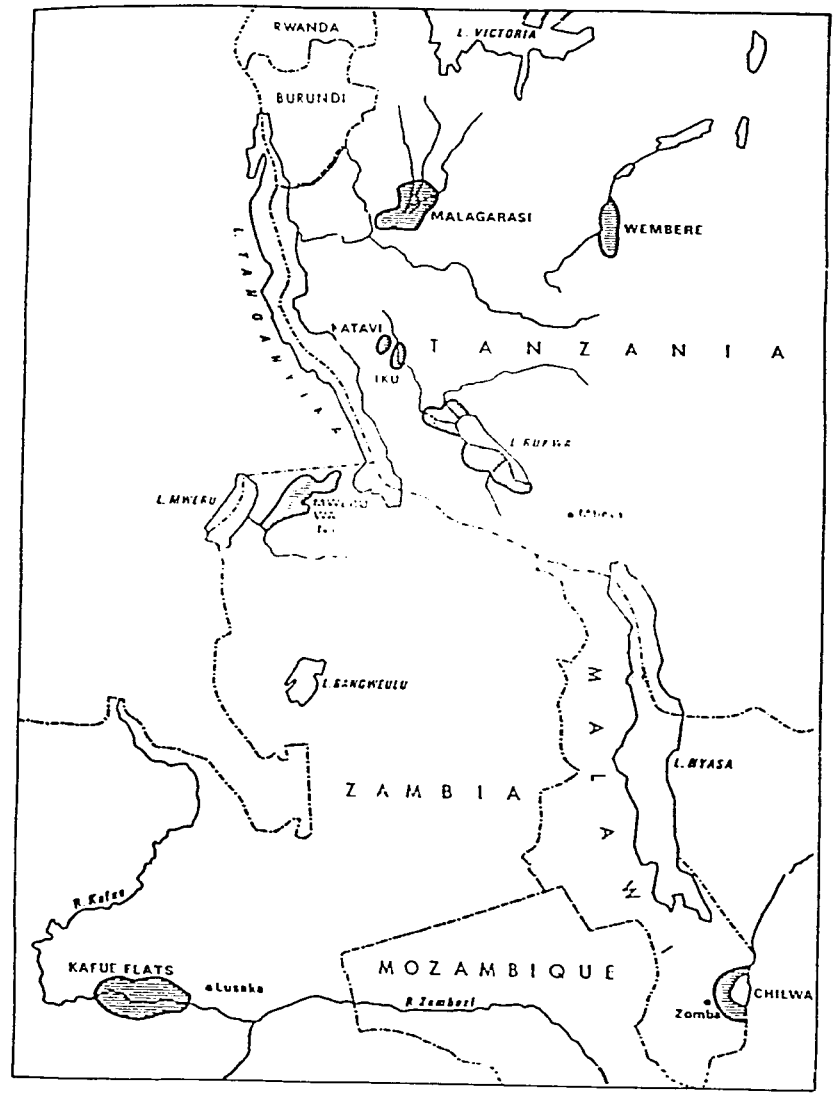

Figure 1. The recognized outbreak areas of the red locust. Shaded areas are those actually or potentially capable of producing swarms. (From Stortenbeker, 1967).

Table 1. Frequency of recorded and probable swarm formation and escape from the recognized outbreak areas of the red locust between 1945 and 1987.

Area	Number of years			
	Swarm escaped	Swarms within outbreak area only	Swarm formation possible without control	Total
Wembere	8	4	5	17
Rukwa	5	7	4	16
Malagarasi	3	3	4	10
Iku/Katavi	2	5	3	10
Buzi/Gorongosa	5	2	0	7
Kafue Flats	0	6	0	6
Mweru-wa-Ntipa	2	1	2	5
Chilwa	0	1	0	1

1960), Symmons (1978) and Materu (1984). Briefly, they are areas of extensive and open grasslands with impeded or closed drainage, consequently, they are subject to prolonged flooding during the rainy season, whereas during the latter part of the dry season, conditions can be extremely hot and dry. Generally, they are well defined areas, and it is here that red locust numbers fluctuate widely from scattered populations to swarm density, even during recession.

Vesey-Fitzgerald (1964) and Materu (1984) reviewed the ecological niches of different stages of *N. septemfasciata* in outbreak areas and reiterated an earlier view (Clark, 1947) that the ecological niche essentially consists of food-shelter and oviposition habitats. Materu (1984) then went on to suggest that it is these two habitat factors which influence the size of the population and, consequently, swarm formation. However, no definite studies have been conducted to determine the extent of this influence. Nonetheless, it is not clear if these are the only factors that influence the numbers of *N. septemfasciata*, and the role of natural enemies in the population fluctuations is not known.

Life cycle

In central and southern Africa *N. septemfasciata* lives in a region with one dry season per year. The wet season begins around November and lasts until May; oviposition coincides with the beginning of the wet season and the eggs hatch about 30–40 days later. The emerging hoppers pass through

6–7 instars, and become adult around February or March. The adults live through the dry season in immature state until the beginning of the rains when maturation is initiated. Mating and oviposition then follow.

Population dynamics

There have been few investigations on the population dynamics of *N. septemfasciata*, particularly in the field. Robertson (1954, 1958) investigated reproduction of the red locust in an outbreak area and Albrecht (1959) and Norris (1959) independently studied the dynamics of laboratory populations, but their investigations have not been corroborated by field studies.

Symmons (1959) and Symmons & Carnegie (1959) studied the effect of climate and weather on *N. septemfasciata* populations, as well as factors that affect breeding. In his studies on influence of climate and weather, Symmons (1959) demonstrated that fluctuations in numbers from one generation to the next are inversely correlated with the rainfall total in the last but one rainy season. In these studies, no mention was made of the influence of biotic factors on population numbers.

More comprehensive work in the field, however, was the study by Stortenbeker (1967) who investigated population dynamics of *N. septemfasciata* in an outbreak area. In addition to corroborating Symmons' (1959) work, Stortenbeker (1967) also showed that reductions of the parent numbers and the fledging process in the filial populations were correlated with rainfall. Stortenbeker (1967) went on to make a number of conclusions on various factors affecting population fluctuations. Significantly, again none of these were biotic factors.

Although it was not the main part of his study, Stortenbeker (1967) made a number of observations on biotic agents that were causing mortality to *N. septemfasciata*. He observed, for example, that Odonata (dragonflies and damsel flies) population numbers appeared to be correlated with the previous year's rainfall and suggested that species of Odonata were probably the most important agents causing fluctuations in the breeding success of the red locust.

Prospects of biological control – a review of natural control agents

Several investigations and reviews of population dynamics of *N. septemfasciata* (e.g. Dean, 1964; Stortenbeker, 1967; Materu, 1984) have

reported a number of natural enemies in its outbreak areas. Thus, Materu (1984) listed the multimammate rat (*Mastomys natalensis*) and mites (Acarina) as egg predators. The hymenopteran *Scelio howardi* Crawford was also found parasitizing the eggs. Predators of hoppers he listed were *Alcimus* sp., *Ommatius variabilis* Engel and *Philodicus nigrescens* Ricardo (as *P. nigripes*) (all Diptera: Asilidae), *Anisoptera* spp. (Odonata) and larvae of Tabanidae (Diptera). Other workers (e.g. Chapman, 1962; Stortenbeker, 1967) have also observed a number of natural enemies of adult locusts. These included parasitic nematodes, a tachnid, *Metacentyia calleti* (Seguy), as *Ceracia nomadacris*), birds, and the fungus *Beauveria* sp.

There are thus, a large number of biocontrol agents causing mortality of different stages of *N. septemfasciata* in its outbreak areas. Unfortunately, even with such a long list of natural enemies, an impact assessment of their influence on populations has never been done. Moreover, insecticides have regularly been sprayed to suppress locust upsurges in outbreak areas and yet the effect of these pesticides on the populations of the biocontrol agents in these areas is also not known. There is, therefore, a challenge for biological control scientists to exploit the special ecosystem of *N. septemfasciata* in their quest to provide a solution to the locust menace.

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NRI; statement of position

J.I. Magor

Natural Resources Institute, UK.

Abstract
The Natural Resources Institute (NRI) has a long history of multi-disciplinary research on problems in tropical agricultural development and is an integral part of the British Government's overseas aid programme. NRI generates income from commissions with the Overseas Development Administration (ODA) as well as from other donor agencies. It has three operational divisions, of which one, Pest Management, contains the Windborne Pest Programme, in which locust and grasshopper research falls. Current research includes pesticide bioassays, grasshopper taxonomy, outbreak forecasting and egg diapause in *Oedaleus senegalensis* (Krauss). The extensive library facilities are particularly strong on locust and grasshopper literature.

Introduction

The Natural Resources Institute (NRI) was established as an Executive Agency in April 1990. It is the scientific arm of Britain's Overseas Development Administration (ODA) within the Foreign and Commonwealth Office.

NRI has a tradition of multi-disciplinary problem solving, research and development which goes back through the Tropical Products Institute, the Land Resources Development Centre, the Centre for Overseas Pest Research, and others including the Anti-Locust Research Centre to the Imperial Institute founded in the late nineteenth century.

The institute forms an integral part of the British Government's

overseas aid programme. Its principal aim is to alleviate poverty and hardship in developing countries by increasing the productivity of their renewable natural resources.

NRI's main fields of expertise are:

1. resource assessment and farming systems
2. integrated pest management
3. food science and crop utilization.

It carries out research and surveys; develops pilot-scale plant, machinery and processes; identifies, prepares, manages and executes projects; provides advice and training; and publishes scientific and development material.

This paper first outlines the structure of British Aid and its interface with NRI, then summarizes the organization of work within NRI, and finally, describes facilities for visitors and NRI's current work on locusts and grasshoppers.

British aid programme

The ODA is responsible for Britain's aid programme to developing countries which in 1990/91 amounted to over £1,600 million. It has development projects and programmes in more than 120 countries. ODA works through its five Development Divisions and with British Embassies and High Commissions overseas to manage the aid programme.

ODA staff have professional advisers dealing with economic and social development, agriculture and renewable resources, engineering, health and population, education and the environment.

Resources are allocated between bilateral aid provided on a country by country basis and multilateral aid, which is Britain's contribution to International Organizations and Institutions.

Research & development in NRI

NRI as an Agency has to cover its full costs with income. This is generated through commissions with ODA's Natural Resources and Environment Department, Geographical Desks and Development Divisions as well as through other donor agencies.

The pattern for Natural Resources Division expenditure is based on a Research Strategy for Renewable Natural Resources. Nine priority areas were identified and are managed at UK Institutes for ODA. The strategy Areas are Resource Assessment and Farming Systems, Agricultural

Engineering, Integrated Pest Management, Food Science and Crop Utilization, Animal Health, Forestry and Agroforestry, and Fisheries.

The three operational divisions at NRI Resource Assessment and Farming Systems, Pest Management and Food Science and Crop Utilization each manages a strategy area within which work is grouped into programmes containing problem areas. The emphasis within these strategy areas is for multi-disciplinary studies and the Institute's 400 professional staff cover a wide range of disciplines, notably anthropology, biochemistry, chemistry, economics, entomology, engineering, food technology, geography, information technology, land use, livestock nutrition, meteorology, physics.

Each problem area covers the whole spectrum of research from generating new knowledge, converting it into new technologies and finally adapting and transferring them for use in developing countries.

Locust and grasshopper research

Work on locusts and grasshoppers comes under the umbrella of the Integrated Pest Management (IPM) Strategy Area in the Windborne Pest Programme. The Strategy Area contains five Programmes aimed at developing IPM systems for controlling pests and diseases of annual, perennial and industrial crops in intensive and subsistence agriculture.

Migrant insects and disease vectors are important epidemic pests and the ability to quantify and forecast their dispersal is a critical factor in limiting crop damage and in developing appropriate control strategies and early warning systems.

Control and forecasting are based on a knowledge of the physiology and behaviour of the pest and their interaction with weather, climate, topography and agriculture. These facts together with a study of historical analogues predict pest incidence and severity in space and time.

The programme uses a wide group of specialists at NRI and in other UK and overseas institutes to investigate the population dynamics, epidemiology, migration, host-plant interactions, pheromones and control of rice pests and vectors, the African armyworm, locusts, grasshoppers and disease agents.

Results are used in other NRI programmes, international institutes, the IRRI rice IPM network and in regional locust organizations and are disseminated in publications and at conferences and workshops. Overseas scientists train with us and come to NRI as visiting experts.

Current locust and grasshopper research

Work on locusts and grasshoppers expanded in response to needs identified during recent outbreaks which matched NRI areas of expertise. Taxonomic revisions of East African grasshoppers are preceding the preparation of an *Identification Handbook*. Insecticides bioassays are in progress and staff have taken part in pesticide and application equipment trials. Information on the recent desert locust plague (1985–89) is being analysed so that the causes of its onset, spread and rapid decline are made available to forecasters. Finally, an examination has started of diapause in the Senegalese grasshopper *Oedaleus senegalensis* (Krauss) as part of a larger project looking at millet production and protection in Mali.

This programme does not address the biocontrol aspects of this workshop but facilities at NRI will be of interest, and an understanding of the art of forecasting grasshopper and locust outbreaks is necessary to those who wish to implement biological control.

NRI facilities

Locust archives

NRI links with locust survey and control go back fifty years to the anti-locust unit selected to hold and analyse reports collected to determine the causes of intermittent plagues. The main body of reports has been microfilmed. It starts in 1929 and ends in 1973 when FAO assumed the responsibility for maintaining the forecasting and central collection of reports. References to locusts from literature before 1929 are also in the archives.

Maps of locust outbreaks which were used in forecasting and other studies are lodged in the archives; *Locusta migratoria migratorioides* (Reiche & Fairmaire) (1928–41) red locust (*Nomadacris septemfasciata* (Serville)) (1925–41) and desert locust (*Schistocerca gregaria* (Forskål)) (1926–73). Temperature, relative humidity and rainfall maps of the desert locust area are available from 1925–37 and there are synoptic weather charts and rainfall maps of this area for 1961–73. Less detailed maps are also available for *Anacridium melanorhodon* (Walker), *Patanga succincta* (Linnaeus), the brown locust (*Locustana pardalina* (Walker)), and *Schistocerca cancellata* (Serville) in Argentina.

NRI library and information services

Resources

NRI library specialises in the literature of tropical agriculture and is recognized as a major world resource in this field. It incorporates the library of its antecedents including the Anti-Locust Research Centre (ALRC), Centre for Overseas Pest Research (COPR) and the Tropical Development Research Institute (TDRI).

The library stock includes journals, books, pamphlets, reprints, theses, annual reports and technical reports in series. Over 2,000 current journal titles are received, many through exchange arrangements.

TRAIS (Tropical Agriculture Information Service) is the name given to the library's in-house computerized bibliographic database. Bibliographic records for literature published from 1980 onwards are held on the computer database. About 11,500 new records are added each year. For earlier literature manual catalogues and indexes have been retained.

The library has on-line access to commercially available external databases, and holds CD-ROM copies of certain databases including Agricola (USDA), AGRIS (FAO) and CAB Abstracts (CABI).

Locust and grasshopper literature was collected comprehensively until about 1980. For this period there are two keys to the literature: a card index arranged by subject and by species which is available for consultation in the NRI library, and Acridological Abstracts, the published abstracts of literature on locusts and grasshoppers which are available from NRI Publications Distribution Officer.

For the period since 1980 the locust and grasshopper collection is less complete. However, it does contain most of the specialist publications and report literature on locusts and grasshoppers of economic importance, but only selected journal articles.

Services available to external users

Visitors – NRI library is open by appointment to visiting readers, for reference purposes only, from 09.00 h to 16.30 h Monday to Friday (excluding public holidays).

Enquiries – library and information services staff are available to answer bibliographic enquiries from external UK and overseas enquiries by letter, telephone or in person. Enquirers should define the subject of their enquiry as precisely as possible. Literature searches can be made for enquirers using the in-house computerized database and manual indexes. In addition the external databases which NRI library holds on CD-ROM can also be searched, but the library does not normally undertake on-line searches on external databases on behalf of external enquirers.

Photocopying – the library can only supply limited numbers of photocopies of articles. Enquirers should first try the library resources within their own country and only ask NRI library to supply what is not available locally. Copies are charged at 10 pence per page (plus VAT) but are free to organizations in developing countries eligible for British aid. Photocopying of copyright material is undertaken strictly within the UK copyright regulations (only one article from a journal or up to 10% of a book).

Remote sensing and GIS

Satellite observations of cloud duration and temperature when suitably calibrated against point surface rainfall measurements can give rapid estimates of rainfall. The TAMSAT system of Primary Data User Systems with micro computers developed in the UK by Bradford University Research Ltd, TAMSAT Research, and the Department of Meteorology at Reading University offer a cheap and robust system for monitoring rainfall using data from METEOSAT.

This system is in operational use by the meteorological services of Morocco (Casablanca); Algeria (Algiers, Tamanrasset); Tunisia (Tunis); Sudan (Khartoum); Jordan (Amman); Ethiopia (Addis Abeba); Somalia (Mogadiscio); Botswana (Gaberone) and by the Desert Locust Control Organization for Eastern Africa (DLCO-EA) in Nairobi. Cost to buy and install, with training is about £20,000.

Another exciting development by this group with Silsoe and NRI is a system to receive NOAA AVHRR data which in the absence of clouds allows vegetation changes to be monitored and from which Normalized Difference Vegetation Indices NDVIs, the greennesss maps used by locust units, are calculated. NOAA is a polar orbitting satellite, and the AVHRR sensor has a swathe width of 1500 km which enables it to complete global coverage every 12 h.

The dish on the receiver has to be steered to the point in the sky at the time the satellite is passing. A manual system has been fully tested and an automatically steered dish is under test. Software for improved geographical correction is scheduled for June. Cost with training is estimated as £25,000 to 30,000.

Publications and training

Results are published and distributed to scientists and plant protection services overseas and training is given.

OCLALAV; biological control of migratory pests, and perspectives in Africa

A.B. Ndiaye

OCLALAV, Dakar, Senegal.

Abstract
The present structure of OCLALAV is outlined, and a description of biological control as it might be applied to migratory pests is described. Work to date on biological control of locusts and grasshoppers is reviewed.

Introduction

OCLALAV, with its headquarters in Dakar, Senegal, is a regional organization for acridid control, begun in 1965 at N'Djamena (Chad) by amalgamating the Communal Organisation for Control of Acridids (Organisation Commune de Lutte Anti-Acridienne – OCLA) and the Organisation for Control of Bird Pests (Organisation Commune de Lutte Anti-Aviaire – OCLAV). The organization's mandate is to undertake control measures against locusts and grain-eating birds. Member countries are: Republic of Benin, Burkina Faso, Cameroun, Côte d'Ivoire, Gambia, Mali, Mauritania, Niger, Senegal and Chad. In 1986, the administrators of OCLALAV decided to restructure the organization, considerably reducing the number of employees.

Biological control

At the end of the Second World War, chemical control was considered as the ultimate weapon for mankind to defend itself against all ills, particu-

larly crop pests. After a decade of spraying, and following the appearance of resistant species and the first signs of a negative impact on man and the environment, people began to consider other pest control techniques. Rather than banning the use of pesticides outright, it proved necessary to forbid the use of first generation (organochlorine) products and to opt for moderate use of second generation pesticides (organophosphates). In almost all countries DDT, HCH and dieldrin were prohibited.

There are many definitions of biological control, the most relevant of which states that biological control 'consists in putting together all appropriate means and methods which can be used by man to make changes in a biological equilibrium in a way which benefits man in a given environment'.

There are many difficulties in applying biological control methods to migratory pests as the pests may be more mobile than their control agent. We will quickly review the few biological control trials carried out in Africa for the control of migratory pests.

Biological control of locusts and grasshoppers

Bacteria

Bacillus thuringiensis Berliner was one of the first bacteria used against insects, but no strains virulent against Orthoptera have yet been found. Some early laboratory work using different bacterial species has been carried out (Stephens, 1959; Stevenson, 1959).

Other infections

Other infective organisms have been tried with variable results. In 1989, a microsporidian called *Nosema locustae* Canning was used in Mali. This protozoan was discovered on *Locusta migratoria migratorioides* (Reiche & Fairmaire) and has already been used in acridid control in North America. Trials have been carried out in the Cape Verde Islands (Lobo-Lima *et al.*, these proceedings) and in Mali but the results have been disappointing to date.

Chemical attractants

Experiments have been done taking into account the well-developed olfactory sense in insects, in order to concentrate and destroy pest species. Until now, very few viable results have been obtained in Africa.

Use of hormones or pheromones

Hormones, especially those which control growth and moulting in insects,

have been used in experiments to disturb moulting patterns or growth of young larvae. It has proved possible to cause atypical moulting or to maintain individuals in the larval state. Pheromones were used because of their attractant powers on mature adults in particular. Hormones and pheromones are specific to arthropods, therefore risks to human health and to the environment are very small.

Unappetizing substances in plants

Trials using sprays made from fruits and leaves of neem, *Azadirachta indica*, have given encouraging results for certain sedentary species of grasshopper and this led to the isolation of azadirachtin, the active ingredient of fruits and leaves of neem. The use of this plant as a reforestation tree shows that its use on a large scale could be envisaged. Since neem is so abundant, it is readily available to all farmers at little or no cost. It has a repellent effect on grasshoppers; other studies on neem seem to show an effect as a growth regulator (see Langewald, these proceedings).

Cultural control

Agricultural control measures (digging up egg-pods and desiccation of embryos) could be achieved, especially with those species of grasshopper such as *Zonocerus variegatus* (Linnaeus), which lay their eggs in groups. The development of such procedures should occur in the framework of an integrated pest management programme.

However, for the time being, the use of chemical pesticides is the only efficient means of grasshopper control.

Other control possibilities

There are a great number of insectivorous vertebrates, such as rodents many species of birds and reptiles. The impact of these vertebrates as auxiliaries in the reduction of insect populations is important naturally, but they cannot be manipulated at will.

Control measures for small vertebrates (grain eating birds)

Bacteria

The impact of bacteria on birds and rodents has been investigated, but no proposals for their use as control agents have been made because of the health risk.

The contribution of predators

Destruction of nests and small dormitories by flights of migrating raptors such as kites, sparrow-hawks and eagles has been observed.

Advantages of biological control and the future for its use in Africa

Biological control has the following advantages:

1. it may be relatively cheap
2. it does not cause resistance as in use of pesticides
3. it can be used where chemical control is unacceptable.

Use of biological control in Africa

Difficulties in using biological control methods should not lead to discouragement, on the contrary, it is sufficient to weigh the advantages against the disadvantages to be immediately aware of the benefit of these methods. Africa is rich in human resources, but it does not have the financial resources to undertake the research which will lead to the perfecting of appropriate technology and to organizing training sessions.

Constraints for biological control in Africa

For the efficient use of these procedures a minimum level of scientific knowledge is necessary. It is also necessary to:

1. know the environment in which one is working
2. study the biology and behaviour of the pest in depth
3. understand the biology and behaviour of host populations
4. carry out much research and training at all levels.

Conclusion

In 1986, 1987 and 1988 unprecedented quantities of pesticides were sprayed in the fight against acridids. Biological control could help to reduce the amounts of pesticide applied and thus conserve the sahelian ecosystem.

We know that when we have mastered the use of biological control this formidable tool will open up the prospect of a great victory over enemies of crops in Africa.

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PRIFAS; notes from PRIFAS on biological control of locusts and grasshoppers

M.H. Launois-Luong

CIRAD-PRIFAS, Département GERDAT.

Abstract

PRIFAS has not so far been involved in work on biological control of locusts and grasshoppers. However, PRIFAS scientists have extensive experience in detailed ecological work on acridids which would be of great value in implementing any biological control programme.

Apart from observations on natural enemies carried out during studies of acridid population dynamics, PRIFAS has not yet undertaken specific research into biological control. Nevertheless, it may be able to contribute some ideas towards consolidating research aims using a global bioecological approach, through simultaneous studies on all aspects of the ecology of individual acridids, of acridid communities and of their natural enemies.

The reduction in numbers of acridids by biological agents occurs in natural conditions through the action of predators, scavengers, parasites and/or pathogens (fungi, viruses, bacteria). The list is very long and particularly concerns major acridid pests where there is potential for a population explosion. To these can be added indirect methods, such as the use of pheromones which encourage individuals to group together, thus making interventions easier, or insect growth regulators, which aim specifically at larvae by inhibiting feeding.

Before control of an organism is undertaken, in-depth knowledge of its ecological behaviour should be available. It is crucial to be able to decode the multi-factorial system which creates situations favourable to population explosions. Knowing when to intervene is the main problem. The answer is not always clear, since so little is known about the bioecology of the pest

and its natural enemies. According to what criteria should the threshold of vulnerability of a given taxon be defined? Should we wait for economic evidence or act at periods of maximum vulnerability, when the organism is already in difficulties due to eco-meteorological problems, for example? Whether to intervene or not, must depend on the ecological characteristics of each species.

Specific situations occur when the organism is at the limit of its natural distribution. The prognosis for the species could be favourable or unfavourable, depending on its ecological flexibility and its capacity for survival under adverse conditions. Once removed from its centre of ecological stability, the organism may become more vulnerable. This is the case with the migratory locust *Locusta migratoria* (Linnaeus) in its gregarious phase, when it overflows from the humid tropical zone. Alternatively, the species may develop a new survival strategy as migratory locusts have done, by becoming sedentary in artificial oases (in Sarir in Libya), in an area far to the north of its normal area of distribution.

Voltinism also influences the chances of success of a control measure. Univoltine taxa such as *Zonocerus variegatus* (Linnaeus) or *Kraussaria angulifera* (Krauss) are targets for which control has longer lasting effects than for plurivoltine species such as *Schistocerca gregaria* (Forskål) or *Oedaleus senegalensis* (Krauss), especially if populations of adults are very mobile and capable of dispersing over very long distances.

There are as many intervention strategies as there are species and one cannot be limited to only two strategies, for example one for locusts and one for grasshoppers.

The synecological framework defines communities of species which colonize the same habitats. Time and space variants are the indices of stability or instability in a particular ecological zone. The specificity of actions of natural enemies is an important point to establish, since control efforts should be targeted towards economically important species which are already in population imbalance. Many observations have been made and studies carried out on the biology and ecology of certain natural enemies. It is to be hoped that taxonomic inventories complemented by already existing bio-ecological data, continue to be updated and made accessible.

Biocoenotic studies define the host ecological niche, by describing vegetation groupings which are true ecological indicators of the environment, and integrating them with static and dynamic components of local or regional ecometeorological events. These will show the likelihood of proliferation, the degree of aggressiveness or simply the possibilities of survival of an external pathogen in its new habitat.

The biogeography of acridids is an important indicator to ascertain the importance and the type of ecological zones where control measures could take place. The type of habitat indicates the likely success of a biological

control measure. Thus, the harshness of the climate of desert regions, where most of the gregarious foci of migratory locusts in Africa are situated (apart from those around the Red Sea), is in itself a major difficulty for the survival and proliferation of a pathogen.

On islands such as the Cape Verde Islands there is a closed system which is favourable for biological control, as long as safety of the agent for non-target organisms is assured. An imbalance introduced into a biocoenosis can last for a long time, due to slow exchanges with the continent and other islands, and will lead eventually to a new equilibrium of the organism. Exploration carried out in different islands revealed a significant population of acridids parasitized by Diptera larvae at the end of the rainy season which leads to speculation about the impact which this natural limiting factor could have on the population. At the same time, trials with *Nosema locustae* on *Oedaleus senegalensis* have not been conclusive, probably because of the great mobility of the adults, which prevented good contamination of the population.

An oasis effect created on a continent by deforestation does not generate such an impervious environment as an island, and exchanges are more difficult to control.

This global approach, ecological, synecological, biocoenotic, biogeographic, of the host and its aggressor, allows us to define the outlines of their respective ecological niches. The cross-checking of their ecology with behaviour will provide the spatial and temporal framework for the optimum moments of vulnerability of the acridid at the same time as those of the optimum harmfulness of its natural enemy. Many questions could be partially answered:

1. what are the encounter strategies of the natural enemies with their hosts? Do they show active identification, interception or attack modes, or are they passive modes using proliferation of the inoculum to increase the chances of encounter?
2. what are the modes of action and eco-meteorological conditions necessary for an optimum expression of their harmfulness?
3. how long will the pathogen remain active or alive, what is the likelihood of proliferation and survival?
4. what are the alternative hosts or the forms of survival if the host is absent?
5. what guarantee is there that they have a specific action and that they are innocuous to the environment?

PRIFAS is ready to invest in this kind of approach, alone or in collaboration, as long as there are visible sources of funding. It is probable that the species of acridids most likely to become the object of biological control, apart from *Zonocerus variegatus*, which is already being studied, are *Kraussaria angulifera*, *Cataloipus cymbiferus* (Krauss), *Hieroglyphus*

daganensis Krauss, *Kraussella amabile* (Krauss), species which are having an increasing economic impact on the Sahel.

Field trials could be complemented by studies in the laboratory in Africa or at Montpellier and in the latter case, in the framework of the proposed Inter-Organism Centre (CIO). This is a specialist research structure which would regroup INRA, CIRAD, ORSTOM, CNRS, ENSAM, USTL, in the context of the international biological control complex of AGROPOLIS, CILBA which includes Australian partners (CSIRO) and Americans (USDA), and which represents the emergence of an accumulation of knowledge and skills in operational biological control.

COUNTRY REPORTS

Congo; the acridid situation in the Congo

G. Bani

Direction Générale de la Recherche Scientifique, Brazzaville.

Introduction

The Congo, with a surface area of 342,000 km^2 of forest and savanna, has a humid tropical climate with an average temperature of 26°C, 85% relative humidity and rainfall of 2,000 mm. The wet season is from October to May and the dry season from June to September. There are approximately 2,000,000 inhabitants in the Congo, whose basic staple is cassava, not only the tubers, but the leaves also, form an important part of the diet.

There are many crop pests and diseases in the Congo. Crop pests identified include acridid species such as the variegated grasshopper, *Zonocerus variegatus* (Linnaeus) which is, at the moment, the only species to have attained the status of economic pest.

Distribution and habitat

Z. variegatus can be found in all regions of the Congo, on the edges of paths, close to villages, vegetable growing areas and cassava agro-ecosystems. It is not found in uncultivated forests and savanna.

Damage

The importance of the damage caused by *Z. variegatus* varies according to ecological zones and crops; in the forest zone of Mayombe, it is the single

most important pest of cassava (*Manihot esculenta*). Damage occurs in the first month after the cutting is planted and usually it is completely destroyed. Farmers may replant two or three times but only cuttings planted later on manage to avoid the ravages of early attack. Nevertheless, next year's attacks are also important, since defoliation by grasshoppers affects farmers who themselves consume the leaves and can also sell them for a high price.

In vegetable growing zones, *Z. variegatus* is a permanent menace to green leafy vegetable production, since it diminishes the commercial value of the product by the damage it causes.

Biological cycle

Research carried out shows that the biological cycle of *Z. variegatus* is univoltine and variable. Hatching can occur from May to August, depending on the location. Oviposition occurs from December to January or a little later. Some hatching has been observed in January/February in certain areas but this is not a bivoltine cycle, since there are two univoltine populations present reproducing at different times of the year. This case is being studied.

Control

Until recently, chemical methods have been used to control populations of *Z. variegatus* around cash crop plantations, coffee (*Coffea* spp.), cocoa (*Theobroma cacao*), citrus (*Citrus* spp.). In cassava plantations man simply harvested what the undesirable visitor left for him. However, we have been able to institute a strategy which allows us to reduce or avoid early attacks on cassava. This consists of staggering the times when cuttings are taken, depending on hatching times. This practice has already been adopted by a few forward-thinking farmers. It is only applicable to cassava and only in the first months of planting.

Suggestions

Control of *Z. variegatus* in the Congo is a real need and, recognizing the limits of chemical control, we think that everything possible should be done to institute a system of control which does not damage the environment and is economically viable. In order to do this, it is necessary to understand

all the biotic factors for population regulation so that we can reinforce the action still further. Thus, specific attention should be given to the parasitoid complex of *Z. variegatus*. We are in favour of all endeavours in this direction and we firmly uphold any initiatives for co-operation in research for biological control agents of *Z. variegatus*.

COUNTRY REPORTS

Mali; notes on the acridid problem in Mali

B. Maiga

Service de la Protection des Végétaux, Mali.

Introduction

Mali has a varied collection of pests: locusts and grasshoppers, birds, rodents, beetles (leaf miners and leaf beetles) which cause considerable damage to crops and crop yields every year. Since they appear so regularly, locusts and grasshoppers are by far the most important of these pests. In fact, since the droughts began in 1970, there have been problems with grasshoppers every year, and this necessitates the deployment of great financial, material and human resources; Mali finds itself obliged to request financial aid in order to finance its plant protection campaigns.

Strategy and control methods used against acridids

In Mali, the Plant Protection Service (PPS) uses the following strategies and methods:

1. Locusts. Preventative control consisting of intensive observation of gregarious foci, with the aim of stopping locusts becoming gregarious and so preventing swarms from spreading and reaching crop zones.
2. Grasshoppers. Since acridids are a semi-permanent scourge throughout the Sudano-sahelian belt, the accent is more and more on preventative control. First, mobilizing farmers to dig up egg-pods in their fields and the immediate surroundings during the dry season and secondly treating larvae at the beginning of the rainy season.

In keeping with its resources, the PPS opted for grass-roots protection of crops, with the farmer as a key element in protecting his own crops and

the officers of the PPS taking charge of remote areas, pasture and uninhabited areas. Farmers are organized into plant protection brigades, trained and equipped to protect village crops. Digging up egg-pods and early control of larvae gives a significant reduction in the use of pesticides. Further control measures are only used if necessary.

The main species of economic importance are:

1. *Hieroglyphus daganensis* Krauss
2. *Kraussaria angulifera* (Krauss)
3. *Oedaleus senegalensis* (Krauss)
4. *Cataloipus fuscocoeruleipes* Sjöstedt
5. *Cataloipus cymbiferus* (Krauss)
6. *Kraussella amabile* (Krauss)
7. *Aiolopus simulatrix* (Walker)
8. *Diabolocatantops axillaris* (Thunberg)
9. *Cryptocatantops haemorrhoidalis* (Krauss)
10. *Ornithacris turbida cavroisi* (Finot)
11. *Pyrgomorpha cognata* Krauss

These species can co-exist at high densities and can cause serious damage.

Area covered by exploration

Observation

All the Sahelian and Saharo-sahelian region is under direct observation by teams of plant protection explorers, these comprise at least 15 exploration teams deployed throughout the region.

Areas treated with chemicals against different pests

During the last five years an average of 516,000 ha per year has been treated in Mali (Table 1). Although acridids are the main targets of treatments, the chemicals affect all pests in treated areas.

Cost of plant protection campaigns over the past five years

The PPS has received funds from the Department of Agriculture, the National Budget, foreign governments and international organizations.

Table 1. Area treated with chemicals 1987–91.

Campaign	Area treated	Area infested
1986–87	595,337 ha	1,000,000 ha
1987–88	330,597 ha	800,000 ha
1988–89	502,098 ha	1,200,000 ha
1989–90	722,492 ha	1,500,000 ha
1990–91	459,471 ha	525,000 ha

National budget

1986–87	121,622,000 CFA Fr
1987–88	150,000,000 CFA Fr
1988–89	250,000,000 CFA Fr
1989–90	310,000,000 CFA Fr
1990–91	297,000,000 CFA Fr

International Organizations – Emergency funds

1986–87	268,453,000 CFA Fr
1987–88	1,102,515,000 CFA Fr
1988–89	1,575,779,000 CFA Fr
1989–90	1,032,685,000 CFA Fr
1990–91	297,000,000 CFA Fr

Conclusion

In Mali, there are a variety of crop pests but acridids are the most important and every year enormous resources are deployed to control their increase. These resources are often greater than the country has at its disposal, hence the plea for urgent help from the international community. To combat these difficulties, the PPS in Mali has opted for a grass-roots approach which means that the farmer must assume responsibility for the protection of his own fields and immediate surroundings. However, enormous quantities of chemical pesticides (more than 200,000 l of liquid chemical and 500 t of powder each year) are still being used with all the risks that this entails for man and his environment. The PPS recognises the dangers, which is why it is turning towards research into and application of

alternative methods, digging up egg-pods, end of season cultivation, proper field and border sanitation, trials of biological products, harvest and trial rearing of egg-pod parasites, etc. This research is aimed at the reduction of the use of chemcial pesticides and is carried out in collaboration with partners such as FAO, USAID, Ciba-Geigy, etc.

COUNTRY REPORTS

Republic of Benin: the acridid situation in the Republic of Benin

A. Coffi

Service Protection des Végétaux, Benin.

Introduction

For the past ten years Benin has undergone wide-ranging attacks by grasshoppers in the north (near the border with Niger), on crops such as maize (*Zea mays*), sorghum (*Sorghum* sp.) rice (*Oryza sativa*) and cassava (*Manihot esculenta*). *Zonocerus variegatus* (Linnaeus) has become a pest of some importance in the south since 1985. It has become necessary to formulate a national strategy against grasshoppers.

1982 saw an unprecedented proliferation of grasshoppers. The FAO collaborated with the government of Benin to spray an area of 20,000 ha in the north from the air. Since then, Benin has treated an average of 6,000 ha of crops and fallow annually.

Organization

Training of extension workers and farmers

There are approximately 20 especially recruited extension officers, who have helped farmers and involved them in control measures.

Exploration

We have found about 15 very common species of grasshopper (Acrididae) of which six are of economic importance:

1. *Hieroglyphus daganensis* Krauss
2. *Kraussaria angulifera* (Krauss)

3. *Oedaleus senegalensis* (Krauss)
4. *Oedaleus nigeriensis* Uvarov
5. *Cataloipus fuscocoeruleipes* (Sjöstedt)
6. *Zonocerus variegatus* (Linnaeus)

Population density during infestations is from 50 to 230 larvae/m^2, counting all species. Pressure from grasshoppers was intense until 1988, when due to exceptionally heavy rains grasshoppers drowned and crops were lost.

In 1989, there was calm, but in 1990 a resurgence took place from those areas which had not been flooded, and from external populations borne in on the winds.

Treatment

Spraying takes place from June to September or October, during the rainy season. The primary target is the population of young larvae in fallow, and in crops damaging growing tips, and for this we use powder formulations (propoxur, carbaryl, fenitrothion, Table 1).

From August to September there follows an intensive treatment

Table 1. Anti-grasshopper activities in Benin.

		North		South
Year	Insecticides used	Quantity	Area treated ha	Area treated ha
1986	propoxur	4.0 t	2,980	6,666
	HCH	1.9 t		
	fenitrothion	3390.0 l		
	dieldrin			
1987	propoxur	2.0 t	6,578	7,964
	γ-HCH	0.5 t		
	fenitrothion	60.0 t		
1988	fenitrothion	25.7 t	6,758	4,712
	propoxur	6.7 t		
	malathion	5400.0 l		
1989	propoxur	13.5 t	6,285	
	carbaryl	11.6 t		
	fenitrothion	0.8 t		
	malathion	1310.0 l		
1990	propoxur	15.3 t	4,614	
	carbaryl	0.5 t		

against advanced larvae and adults with ULV formulations (malathion, fenitrothion, Table 1).

Evaluation of treatments takes place to see how effective they have been and whether isolated pockets need to be treated again.

Future aims

- Organization of farmers into farmer brigades
- Providing brigades with means of treatment
- Reviving observation posts in the north
- Training

Zonocerus variegatus – the variegated grasshopper

This grasshopper lives in the southern part of the country and attacks maize, cotton (*Gossypium* spp.), cassava and teak (*Tectona grandis*), especially in the long dry season, causing defoliation and bark-stripping on cassava. The longer the dry season, the worse the threat. From 1986–89 there were three control campaigns over 20,000 ha and due to this successful treatment the situation is calm at the moment.

There is still much work to be done (real damage, effect of attacks on tubers), since although *Z. variegatus* is not a problem now, there are still ecological zones favourable to grasshopper proliferation in areas of natural forest surrounded by planted forest, as in the Lama forest.

Complementary to these activities research work has been done in the laboratory and in the field.

Laboratory

The influence of neem oil on different stages of *Zonocerus variegatus* larvae was investigated, at 5 l/ha and 10 l/ha.

Results: neem oil inhibits moulting and affects the antennae.

In the field

Z. variegatus damage was simulated on cassava.

Results: the damage made no significant difference in yield.

Conclusion

In Benin, we have used only chemical insecticides against locusts and grasshoppers, which may well present great risks for the environment, man and other animals.

The insecticides used only limit the damage done to crops, without any respect for non-target species.

Although the use of insecticides is in response to a pressing need for the elimination of insects in crops, when possible long- and short-term harmful effects are taken into account, it becomes imperative to identify other approaches which can take the protection of nature into account.

COUNTRY REPORTS

Senegal; grasshopper and locust control in Senegal

A. Niassy

Directorate of Plant Protection, Dakar, Senegal.

Abstract

In Senegal, grasshopper control represents 80% of pest control activities organized by the Directorate for Plant Protection (DPP) in collaboration with OCLALAV and FAO amongst others. Until now, mainly chemicals have been used for control purposes, but now different ideas are being developed, more extension workers are receiving training and more farmers closely monitored. Thus, from large-scale spraying by helicopter, the trend is towards small scale interventions, even by the farmer himself. This new outlook means that we will soon be in a position to introduce biological control methods in an experimental form.

Locust control strategies

Basic findings

In order to define the strategy for the next year, we have to examine population dynamics from preceding campaigns in conjunction with climatic conditions and the results of exploration for egg pods during the previous season.

There are two main groups in locust and grasshopper populations.

Migratory grasshoppers

These move in relation to the intertropical convergence zone (ITCZ). *Oedaleus senegalensis* (Krauss), for example, often causes major damage to cereal crops in the north of Senegal. The behaviour of this species is well known since it has been extensively studied by researchers from PRIFAS. Control begins by observing the ITCZ and studying vegetation maps to predict or locate outbreaks. If the results of this method seem positive, the

model will become the main tool in observation of the species. The Pest Forecasting Bureau at the Crop Protection Service started to use the PRIFAS biomodel in 1990.

Sedentary species

These can be divided into two main groups:

1. Wet season grasshoppers; univoltine with embryonic diapause (*Kraussaria angulifera* (Krauss), *Cataloipus cymbiferus* (Krauss), *Hieroglyphus daganensis* Krauss, *Acorypha* spp., etc.). These insects hatch in July/August and become adult by mid-September. Diapausal eggs are laid at the end of September/ beginning of October. Density and damage can be quite substantial depending on the area. During the 1990–91 campaign 261,601 ha were treated (Table 1).

2. Dry season grasshoppers; univoltine with either adult diapause (*Ornithacris turbida* Walker, *Diabolocatantops axillaris* (Thunberg), or egg diapause (*Zonocerus variegatus* (Linnaeus)), or bivoltine with adult diapause (*Aiolopus simulatrix* (Walker), *Acanthacris ruficornis* (Fabricius)). These attack vegetable and fruit crops. *O. turbida* and *A. ruficornis* have become endemic in the Nioro area and they cause significant damage to cashew tree (*Anacardium occidentale*) plantations; sometimes the whole crop can be lost. Spraying with insecticides is effective but does not solve the whole problem.

Table 1. Summary of the results of treatments in the 1990–91 campaign.

Type of intervention	Area treated (ha)			Proportion of area with acridids (%)
	Acridids	Others	Total	
1. Ground-based applications				
Village committees	30,519	16,309	46,828	65
DPP	198,938	34,248	233,186	85
Total terrestrial	229,457	50,557	280,014	82
2. Aerial applications				
ECOFORCE (French Cooperation Project)	32,144	–	32,144	100
Total aerial	32,144	–	32,144	100
Overall total	261,601	50,557	312,158	84

Source: Anon. (1991b)

Strategies

Chemical control of wet season grasshoppers

The main methods of control are:

1. early intervention (farmers' level) in small areas concentrating on groups of larvae in fields using powder and emulsifiable concentrates
2. intervention on the ground over larger areas on fallow land and in forests; control of adults is done using ULV sprayers and emulsifiable concentrates
3. aerial intervention when the infestation is general and cannot be treated on the ground.

The type of control strategy used depends on the following points:

1. the importance of the crop to be protected
2. the nature and developmental stage of the pest
3. surface area infested and location
4. resources available.

Thanks to the efforts of the state in conjunction with the European Community, the Directorate has large human and logistical resources at its disposal.

Human resources

At Directorate level there are 273 personnel. In addition, there are village pest control committees, extension officers and experts from the international community (computer specialists, aeroplane mechanics and pilots). Local labour may be employed as necessary.

Material resources

201 vehicles for application, liaison, exploration and maintenance, 31 of them with proper equipment. 900 pairs of boots, 900 goggles, 900 masks and 99 pairs of gloves are available at the ten regional Plant Protection Inspectorates.

During extensive infestations in certain regions close to Dakar, the French Cooperation Project (ECOFORCE) allocated a helicopter to Senegal for 90 flying hours.

Ground spraying

This was done by motorized units from the DPP and anti-locust village committees. The surface area treated was 229,457 ha, with 198,938 ha by the DPP and 30,519 by village committees. There was about 90% mortality in 48 hours.

Aerial spraying

This was carried out by helicopter on areas of massive infestation of five species of grasshoppers over a total of 32,144 ha in different regions. Using fenitrothion there was 81.8% mortality after 24 hours and 90.4% after 48 hours.

A total of 312,158 ha was treated. Treatments affected grasshoppers and other pests (hairy caterpillars, scale insects and cantharid beetles) and birds.

The total of chemicals used was as follows:

393 t of powder
14,469 l of emulsifiable concentrate
169,390 l of ULV formulations

The pesticides were fenitrothion, carbaryl, chlorpyriphos, malathion and lambdacyhalothrin (Table 2).

It is obvious from these figures, which reflect a fairly calm period of activity, how expensive locust and grasshopper control can be. Chemical control needs vast financial and material resources and risks polluting the environment. Therefore, other methods of control should be considered, especially now that environmental protection has become so important world-wide.

Control using poisoned baits

This has certain advantages for grasshopper control if well thought out. The surface area treated can be considerably smaller and can affect winged adults and larvae in the dry season or at the beginning of the wet season, using a base (bran) provided by the farmer himself. This method involves the farmer in the monitoring and protection of his own farm. In Senegal, control with insecticide baits using millet bran with carbaryl and certain feeding stimulants has been effective (82% mortality) against *Ornithacris turbida*. It may be worth using against *Zonocerus variegatus* and *Aiolopus simulatrix* which threaten crops in southern and northern areas of the country respectively.

Trials are in progress to assess the effectiveness and economic impact

Table 2. Summary of nationally approved chemical insecticides.

Product	Application kg (wp) or L (ec and ULV)			National total
	DPP	Village committees	Aerial	
Wettable powders				
Fenitrothion 3%	13,997	339,794	–	
Propoxur 2%	–	7,690	–	
Propoxur 1%	–	800	–	
Carbaryl 5%	–	9,100	–	
Chlorpyriphos 5%	9,395	12,655	–	
Total wp	23,392	370,039	–	393,431
Emulsifiable concentrates				
Fenitrothion 50	7,151	2,502	–	
'Sumicombi 30'*	1,726	3,089	–	
Total ec	8,877	5,591	–	14,468
ULV formulations				
Fenitrothion 500	44,086	284	–	
Fenitrothion 250	26,565	–	29,930	
Malathion 95%	15,808	–	–	
Chlorpyriphos**	23,031	–	–	
Lambdacyhalothrin 1%	3,200	–	16,792	
Chlorpyriphos 24	9,695	–	–	
Total ULV	122,385	284	46,722	169,391

*50:1 mixture of fenitrothion and fenvalerate
**Concentration not specified

of this method. We will try to use these baits containing pathogens, as well as chemical insecticides, more often in future.

Biological control

This method may be useful against sedentary species (see above) since they develop in relatively small areas and it should be possible for natural enemies and pathogens to maintain high enough levels to control pest populations. Studies on the impact of natural enemies on egg pods of *Cataloipus cymbiferus* (Anon., 1991a; Niassy, in press) have shown that the level of attack can be as high as 60% on egg pods. On *Kraussaria angulifera* and *Hieroglyphus daganensis* 11% were affected three months after the start of the rainy season.

Predators of egg pods include tenebrionids such as *Pimelia senegalensis* Olivier, *Vieta senegalensis* (Klug) and carabids such as *Styphlomerus* sp. etc. *P. senegalensis* larvae are often seen on *K. angulifera* egg cases. Comprehensive studies on insect enemies have been done by Greathead (1963), Popov (1980), Van der Valk (1990) and Popov *et al.* (1990). Van der Valk's study, carried out in Senegal, stressed the abundance of certain locust enemies and their susceptibility to chemical insecticides.

Work has also been done on pathogens. Prior & Greathead (1989) discussed the possibilities in this area. In Senegal, investigations on the use of pathogens such as *Nosema locustae* Canning have been done at DPP with the help of USAID. These trials included different species of grasshopper including *Zonocerus variegatus* and *Oedaleus senegalensis* (Fishpool, 1982).

Growth regulators have also been used against locusts and the results were interesting, but methods are still experimental. However, the pilot project (Niassy, 1990) showed that they can compete with chemical insecticides such as fenitrothion and chlorpyriphos.

Two new projects are about to start. The first will deal with biological control using entomopathogens and is financed by the governments of the Netherlands, USA, Canada and UK. It will concern itself with Sahelian countries and is based at IIBC (UK), IITA (Benin) and DFPV in Niger (see paper by Prior *et al.*, these proceedings). Senegal will participate in this project.

The second project is a study of the impact of pesticides on the environment, with a sub-programme dealing with useful entomofauna. It will be based in Senegal and is financed by the Netherlands and coordinated by FAO and the National Plant Protection Service.

Senegal is ready to lend every effort towards developing biological control.

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COUNTRY REPORTS

Sudan; the situation of desert locust, tree locust and grasshoppers in the Sudan (1990–91)

A. Abdalla

Locust and Grasshopper Control Unit, Plant Protection Directorate, Sudan.

Abstract
The status of the two main locust species, *Schistocerca gregaria* (Forskål) and *Anacridium melanorhodon* (Walker), and of grasshoppers in the Sudan in 1990 and 1991 is briefly reviewed.

Introduction

During 1990 in the Sudan, rainfall was generally below average and this meant that breeding conditions for locusts and grasshoppers were not favourable. In fact, survey operations in summer breeding zones between latitudes 12°N–18°N, showed that no locusts were present.

Desert locust, *Schistocerca gregaria* (Forskål)

During the summer campaign, all areas were calm and only a few scattered individuals were found in three localities in the Central region of Sudan during August, at a density of one locust/km^2. Both ground and aerial surveys produced the same conclusions. The main crops in the surveyed areas were vegetables, millet, and sorghum, and no damage was reported.

Tree locust, *Anacridium melanorhodon* (Walker)

The same survey teams were used for tree locust as for desert locust and here too the local population reflected the low rainfall with tree locusts

concentrated in relatively high rainfall areas. In June, ground surveys in Central region indicated some threat by tree locusts to orchard crops. In July, in Darfur the total infested area was about 1,700 ha with a medium density of locusts and in north Kordufan the infested area was 100,000 ha, but most swarms dispersed since low rainfall made conditions unsuitable for egg laying. In September in Kadugli, more rain encouraged egg-laying which resulted in a high density presence at 60–70 individuals per tree, of 2nd–5th instar. The area infested from August to October 1990 was 28,660 ha, with less than half affected by adults.

During this period, 3,000 ha were sprayed aerially and 17,000 ha on the ground using propoxur dust 1%, diazinon EC 60% and fenitrothion 96%.

Grasshoppers

After some rainfall in July, grasshoppers (1–5 individuals/m^2) were seen in Darfur and Eastern region. In August, after more rain, there were outbreaks in Darfur of several species of grasshopper, attacking mainly millet. In one area of about 4,000 ha, up to 80% damage to millet and sorghum was noted with a grasshopper density of 20–70 individuals/m^2. Propoxur was sprayed.

1990–91

During the winter breeding season the desert locust situation was generally calm. The Tokar Delta is the most ecologically suitable habitat for the desert locust to breed, since it is flooded annually. The area cultivated here is usually about 30,000 ha and can be as large as 80,000 ha, but this year was exceptionally small with only about 85 ha flooded – a completely new phenomenon.

In November, some scattered individuals were found in the Tokar Delta over an area of 200 ha at a density of one individual/ha. The crops cultivated here are sorghum and millet. All other areas were found free from locusts. The vegetation varied from very dry to green, and included some favourable ecological breeding conditions. During December and January there was still no problem and up until March no gregarious activity was reported, so it seems likely that the calm situation regarding the desert locust will persist throughout 1991, despite the likely presence of scattered adults in the traditional breeding grounds of the Sudan.

Discussion: precis

Dr Johnson congratulated Dr Ndiaye on his talk, but asked why, in Dr Ndiaye's opinion, the *Nosema* trial in Mali in 1989 did not work, since it was not a temperature problem as he had been present when frozen spores arrived from Evans Biocontrol. Dr Ndiaye and Dr Knausenberger were agreed that it was a logistic problem.

Dr Bateman commented to Captain Kitenda that his comments on the involvement of farmers and other practical realities were taken very seriously. Could Captain Kitenda help make laboratory studies more realistic by suggesting what might be typical temperatures during spray operations? Captain Kitenda replied that temperatures vary between 10°C and 20°C in the mornings, and that between 07.00 and 10.00 h they may be as low as 8–12°C in some areas.

Dr Niassy commented to Mr Mouhim that a figure of six million litres of chemicals used during the campaign against locusts seemed huge when one considers the risks and the means necessary to put this into practice. Could Mr Mouhim give some information about the logistic framework and equipment necessary on the one hand and on farmer participation on the other. Mr Mouhim responded that the fight against locusts used land based resources, but the greater part of the intervention was from the air. The application was at a dosage of between 0.8 l and 1 l of ULV product/ha. Farmers were especially valuable in warning us and this is what they have mostly been trained to do. Concerning the environment, Dr Ndiaye spoke of the ECOTOX project in Senegal which is concerned about the effects on the environment of chemicals used in the fight against locusts.

Dr Oomen commented to Dr Zelazny that the number of activities described which are aimed at finding and developing biological control agents is impressive. Many questions to be studied are, however, easily

comparable in the different research institutes involved, (e.g. surveying, isolating, bio-assay, formulation, application). In order to obtain results efficiently, considerable co-ordination and co-operation are very desirable. How does the EC provide for and assure such co-operation? Dr Zelazny responded that the co-ordination of research activities is a very important aspect of the CEC project. At the moment, such co-ordination involves mainly identification, processing of collected material and exchange of pathogens to establish standards during testing. The main aim at this stage is to search for and find new and promising disease agents. If the work in the future concentrates more on application and formulation, such co-ordination will have to be intensified.

Dr Ba-Diallo asked Dr Zelazny whether he would welcome collaboration with the newer research institutes from the Sahelian region. Dr Zelazny replied that GTZ has offered fellowships to four people, but it is difficult to find people to go to Germany and funds were very tight.

Mr Shah asked Dr Raina what microbial agents should be used in the pre-gregarization phase of desert locust and how could they be used in the field given that they would need to be easily applicable, persistent and cover an extensive area. Dr Raina responded that biological agents which can perpetuate in the harsh environment of the desert locust through transovarial transmission and which are sustainable with proper formulations in the field are the most preferred. Mr Shah further asked Dr Raina what steps had been taken to ensure that desert locust in culture at ICIPE are solitarious as claimed. Dr Raina responded that morphometric measurements such as femur size, body length, elytra size, etc., had been used, and that biochemical markers using SDS PAGE of haemolymph proteins is under investigation. Dr Johnson asked Dr Raina whether the data on host specificity and host range changes in *Nosema* referred to in his talk had been published; Dr Raina replied that the work was not published, but that a draft article was available for those interested. Dr Johnson commented that enormous quantities of inoculum would be required to treat all recession areas, and that baits would need to remain palatable for many years.

Panel discussion

Dr Cunningham began the discussion, saying that most farmers and the general public are familiar with chemical pesticides and how they work but not with biological control agents. He was of the opinion that 98% of the general public want biological control and that we need training for both farmers and the public, but that we should be careful not to oversell the capabilities of biological control. There should be co-ordination to implement the research which has already been developed. Many technical reports have been produced and implementation teams set up to use and implement the technologies.

What about field implementation? Dr Goettel urged that caution was needed not to push the research too much before it was ready, because of the danger of raising expectations unduly.

Dr Magor discussed a donor's point of view. Donors want results because they see so much research work published. We have a problem now and we need to do something about it now. Maybe we would get better results later on when much more research has been done, but in the meantime we need to go forward with what we have.

Dr Krall felt it was important to involve the farmers wherever possible and collaborate with them in the form of farmer brigades and with the plant protection services. Some basic research still needs to be done in laboratories. Where there are no farmers as such in the recession areas, monitoring and control will have to be done in collaboration with plant protection services. Various people stressed the need to demonstrate safety in the laboratory and efficiency in the field. We should not at this stage promise viable biological control agents to the farmers.

Section II

Agents for Biocontrol

Natural enemies of tropical locusts and grasshoppers: their impact and potential as biological control agents

D.J. Greathead

International Institute of Biological Control.

Abstract

There are numerous records of natural enemies of Acridoidea but few in-depth studies on natural mortality of tropical species. These chiefly relate to the desert locust, *Schistocerca gregaria* (Forksål), African grasshoppers, *Zonocerus variegatus* (Linnaeus) and *Oedaleus senegalensis* Krauss), and the Australian plague locust, *Chortoicetes terminifera* (Walker). The principal groups of natural enemies – vertebrate and invertebrate predators, insect parasitoids, parasitic nematodes and pathogens – are reviewed with emphasis on their importance as mortality factors and potential for manipulation as biological control agents. The conclusions of population dynamic studies on the impact of natural enemies are summarized and it is shown that there is a consensus that natural enemies are unable to prevent locust outbreaks but can provide a useful constraint on the rate of population increase and may hasten the collapse of plagues. The possibilities for biological control are discussed and it is concluded that there is little prospect for classical biological control of mobile locusts but that there may be some opportunities for introduction of insect predators for suppressing static grasshopper populations. Augmentative release programmes are not considered feasible. However, the most promising approach to biological control appears to be the development of pathogens, particularly those fungi which can be cultured *in vitro*, as biological pesticides.

Introduction

There are numerous records of natural enemies of locusts and grasshoppers beginning with the monumental study of Künkel D'Herculais (1893–1905)

in Algeria. The natural enemies which have been recorded include predators, parasitoids and pathogens. Many of those recorded are rare or incidental but there remains a formidable list of potentially important mortality factors to be evaluated.

Insect natural enemies were reviewed and catalogued by Greathead (1963). Information on arthropod and nematode natural enemies in America north of Mexico was summarized by Rees (1973) and lists of natural enemies of tropical species are included in *The locust and grasshopper manual* (COPR, 1973). Knowledge on the pathogens of grasshoppers has been reviewed recently by Streett & McGuire (1990), and Prior & Greathead (1989) have provided an evaluation of the various groups as potential biological control agents for tropical locusts and grasshoppers. There are also some important studies on particular species of natural enemies which are cited below in discussions on these species.

Less information is available on the impact of natural enemies on populations of Acridoidea, especially in the tropics. Studies on populations of the desert locust, *Schistocerca gregaria* (Forskål), were carried out during the 1950–62 plague by scientists of the Anti-Locust Research Centre and the Desert Locust Survey (e.g. Stower *et al.*, 1958; Ashall & Ellis, 1962; Stower & Greathead, 1969). This and other work was summarized by Greathead (1966a) who showed that although the impact of natural enemies is considerable (Tables 1–3) and may reduce the population growth rate of gregarious populations from 11 per generation to 6–8.5 (Table 4), natural enemies are incapable of preventing outbreaks although they may contribute to the collapse of plagues.

The only other relevant studies in Africa are those of Chapman & Page (1979) on *Zonocerus variegatus* (Linnaeus) and by Cheke *et al.* (1980) and Popov (1980) on egg mortality of *Oedaleus senegalensis* (Krauss) (Table 5). Studies have also been made in Australia on the population dynamics of *Chortoicetes terminifera* (Walker) (Farrow, 1977, 1982a, 1982b; Table 6).

Table 1. Estimates of egg mortality of gregarious desert locusts in north-eastern Africa and the Red Sea basin.

Cause	Mortality
Stomorhina lunata (Calliphoridae)	<90%
Systoechus spp. (Bombyliidae)	<30%
Trox procerus (Trogidae)	<10% (75%)*
Fungi, etc.	<30%
Inviability	c. 10%
Hatching failure	c. 4%

*On Saudi Arabian coast only.

Table 2. Estimates of parasitism of nymphs and adults of the desert locust in north-eastern Africa.

Cause		Mortality
Trichopsidea costata (Nemestrinidae)		<30%
Blaesoxipha spp. (Sarcophagidae)	in *gregaria*	<5% – <45%
	in *transiens*	<40%

Table 3. Examples of bird predation on desert locusts in north-eastern Africa.

Bird	Population type	Daily loss
Marabou	swarms	650,000
White stork	swarms	200,000
Eagles	swarms	25,000
Raven	hopper bands	168,000
Hornbill	hopper bands	140,000
Wheatear, etc.	*transiens*	1,200
Kestrel	*transiens*	1,000

Table 4. Desert locust multiplication rates in north-eastern Africa.

Rates of increase	*solitaria*	*transiens*	*gregaria*
Potential rate of increase	65+		50
Actual rate of increase:			
a. without natural enemies	16.5		11
b. with natural enemies			
i. Somali Peninsula	–		6–8.5
ii. Red Sea coast	–	0.03	

Data from Greathead (1966)

This review discusses the important groups of natural enemies in relation to their value as mortality factors and their potential as biological control agents with particular reference to tropical Asia and Africa.

Principal groups of natural enemies

Different suites of natural enemies attack the egg and post-embryonic stages, but there is no useful distinction between those of the nymphs and adults, thus they are best treated as two categories.

Table 5. Mortality of Senegalese grasshopper eggs.

Cause	Niger[a]	Oman[b]
Systoechus sp. (Bombyliidae)	11%	–
Xeramoeba oophaga (Bombyliidae)	4%	2%
Mylabris sp. (Meloidae)	3%	0.5%
Pimelia sp. (Tenebrionidae)	40%	–
Tenebrionid	–	11%
Unidentified	–	27.5%

Data from Popov (1980)
a. means of 104 samples.
b. single sample.

Table 6. Mortality rates for the Australian plague locust during 1971–4.

Cause	Rate (range)	Remarks
Unrealized natality	33.5 (7–57)	minor influence of gregarines
Egg mortality	27.2 (7–60)	83% due to *Scelio*, ants, etc.
Hopper mortality	30.4 (12–58)	mostly rainfall, some predation
Dispersal	6.8	net loss/gain over all generations
Adult mortality	1.5 (<1–11)	parasitoids and mermithids
	—	
	99.4	

Data from Farrow (1982a) means of 17 generations

Natural enemies of the egg stage

All locusts and grasshoppers, except a few specialized species, oviposit in the ground and deposit a batch of eggs which are protected by secretions from the accessory glands – referred to as an 'egg pod'. The secretions form a 'froth plug' which blocks the hole above the eggs and provides both protection from predators and an escape route for the hatching nymphs. In those species with diapausing eggs the secretions may also form a hard capsule of cemented soil around the pod and even occasionally a cap at the soil surface which provide further protection from predators.

Egg predators

Many of the insect natural enemies of eggs consume all or part of the eggs in an egg pod and remain in it throughout their larval life. Thus, they behave more like parasitoids than predators and so are treated as a separate category from conventional predators which move from egg pod to egg pod.

Diptera: Bombyliidae

Bee-flies of the genera *Systoechus* and *Anastoechus* are usually associated with egg pods in North America, Africa and Eurasia. Other genera also contain species specialized as egg predators, e.g. *Xeramoeba oophaga* (Zhakvatkin) on *O. senegalensis*, or sometimes in limited geographical areas, e.g. *Cytherea* spp. in the Mediterranean Basin and Central Asia. They are frequently the most important cause of egg mortality. They are not host specific but individual species attack either non-diapausing egg pods (without hardened walls) or diapausing egg pods which have hardened walls (Greathead, 1963).

The eggs are minute, thin-walled and sticky and are coated with sand in a special chamber formed by the invagination of the posterior abdominal segments which is filled prior to oviposition. The sand covered eggs are ejected onto the ground or into irregularities in the soil surface and the tiny triungulin-like first instar larvae wander in search of their prey. After completing feeding the larvae become dormant until pupation is triggered by a weather event. In the arid parts of Africa this is a rain storm which wets the soil (Hynes, 1947), thus synchronizing emergence with the appearance of a flush of flowers on which the adults feed. Mating requires a large space for courtship flights. This and the fragility of the adults makes breeding of bee-flies in captivity extremely difficult and this has only been achieved rarely and only on an experimental basis (Du Merle & Delpech, 1973).

Diptera: Calliphoridae

Stomorhina lunata (Fabricius) is an unique egg predator within the family Calliphoridae. It is widespread in Africa (except West Africa) and its distribution extends northwards into the Mediterranean Basin and eastwards to the Indus River. Although it is a major predator on egg pods of gregarious desert locusts, it has not been recovered from the egg pods of solitary locusts and grasshoppers so that its alternative hosts in the absence of locusts remain a mystery.

Oviposition takes place into the froth plug and the larvae move down into the egg mass to feed, movement within the egg mass causes extensive damage and ensures infection by fungi and bacteria so that a single larva may cause the destruction of an entire pod. There is no diapause and adult

predators emerge shortly after the nymphs from undamaged egg pods. The adult flies feed on flowers, consuming both nectar and pollen, which is essential for maturation. Flies congregate in large numbers around ovipositing swarms and there is circumstantial evidence that the adults migrate and are thus brought into areas where locust breeding is likely.

Breeding has been carried out on an experimental scale on hen's egg yolk without difficulty (Greathead, 1962).

Coleoptera: Trogidae

Beetles of the genus *Trox* are usually considered to be scavengers but two species have been identified as egg predators, one in southern South America (*T. suberosus* Fabricius) and one in Africa-Arabia (*T. procerus* Harold). On the Red Sea coasts the latter is often an important egg-predator of gregarious desert locusts. Adults burrow in the ground to oviposit near egg pods and the larvae feed indiscriminantly on the eggs. Adults are attracted to ovipositing locusts and feed on cadavers (Roffey, 1958).

Coleoptera: Meloidae

Larvae of *Mylabris* spp. (Africa and Eurasia) and *Epicauta* spp. (North America) prey on eggs of locusts and grasshoppers in most regions but usually their numbers are low and they do not appear to have a significant impact. The numbers of adults may be greater than seems reasonable to account for the number of larvae observed in egg pods, suggesting that there may be other undetected hosts. The life cycle is complex and exhibits hypermetamorphosis. The adults lay large numbers of minute eggs (like the Bombyliidae) which hatch into triungulin larvae. These seek egg pods in which they feed and pass through three instars, as a 'caraboid' and then two 'scarabaeoid' larvae, before moulting into a resting stage, the 'coarctate' larva. In the arid tropics further development seems to require rain. Then there is a further moult, into a 'scolytoid' larva, before pupation. The adults feed on flowers and some species are important pests of crops, especially beans.

Miscellaneous

Egg pods are also attacked by generalist insect predators living in the soil. The most important of these are larvae of Asilidae (Diptera), and Carabidae and Tenebrionidae (Coleoptera). These predators may cause important mortality and can be present consistently at oviposition sites of particular species or at particular localities. However, none of them seems to be dependent on locust and grasshopper eggs for their survival.

Egg parasitiods

Hymenoptera: Scelionidae

The only true parasitoids of the eggs of Acridoidea belong to the genus *Scelio sensu lato*, excepting a few records of chalcidoids from some very specialized grasshoppers which oviposit above ground, e.g. *Oxya* spp. which lay between the leaves of rice plants above the water level.

The African species of *Scelio* were revised by Nixon (1958). Adults penetrate the froth plug to reach the eggs in which they lay their own eggs. The developing stages remain within the chorion until the emerging adult cuts its way out.

There does not seem to be any host specificity but there is a tendency for particular species to be reared from particular hosts (Irshad *et al.*, 1978), possibly egg size and habitat are the chief determinants of host selection. Although *Scelio* spp. can be abundant in grasshopper eggs they are rare or absent from the egg-fields of gregarious locusts in Africa and Asia. However, in Australia *S. fulgidus* Crawford can achieve very heavy mortality (over 90%) of eggs of *Chortoicetes terminifera* in declining populations (Farrow, 1977). Some authors claim to have detected a tendency to density dependence in levels of parasitism but there is often extreme patchiness of attack.

Some species exhibit phoresy and Farrow (1981) has presented evidence that *S. fulgidus* migrates on air currents in such a way that it will be transported to the same areas as migrating *C. terminifera*.

These parasitoids are easily cultured on grasshopper eggs and *S. pembertoni* Timberlake is, so far, the only insect parasitoid to have been successfully introduced as a biological control agent of a grasshopper – against *Oxya chinensis* (Thunberg) in Hawaii (Clausen, 1978).

Pathogens

Investigators studying egg mortality frequently report significant losses due to 'mould' but there have not been any studies which have identified the causal agents. The record of *Cunninghamella* sp. as a pathogen by Daguerre (1937, cited in Roffey, 1968) is dubious since this is a common genus of saprophytes in soil. However, a true pathogen, *Paecilomyces lilacinus* (Thom) Samson, has been isolated from eggs of *Segestidea* sp. (Tettigoniidae) in Papua New Guinea (Prior & Greathead, 1989).

Natural enemies of the post-embryonic stages

Predators

Nymphal and adult Acridoidea are preyed on by a large number of vertebrate and invertebrate predators most of which are generalists. Gregarious locust populations are particularly attractive concentrations of food, and both swarms and hopper bands are often followed by predators, notably migrant birds and sphecid wasps. The only quantitative studies are to be found in the papers cited above but there are also some useful observations scattered in the literature which are cited below in discussing the contribution of particular groups of predators.

Terrestrial vertebrates
Mammals (small carnivores, insectivores and rodents) and reptiles (particularly lizards) are frequently reported as predators but there are no quantitative assessments in the tropics except the study of a *transiens* population of desert locusts in Ethiopia (Stower & Greathead, 1969) and on that occasion they were of negligible importance.

Birds
Predation by birds can be significant (Table 3) and there are reports of birds congregating on small hopper bands and remaining with them until the population has been completely eliminated. This behaviour has been reported both for resident species (passerines, hornbills, rollers, etc.) attacking early instars (Huddleston, 1958, and references therein) and also for storks, especially migrant populations of European and Abdim's storks, attacking bands of older instars and swarms (Smith & Popov, 1953). Raptors have also been credited with causing substantial mortality (e.g. Smith & Popov, 1953) many of these are also migrant.

Migrant birds from the Palaearctic Region, including many of the important predators cited above, arrive in the winter breeding areas of the desert locust and remain there throughout the season (Moreau, 1972) and are thus able to exploit locusts and grasshoppers breeding at this time, but are absent during the summer breeding period and thus insects breeding at this time probably suffer much less bird predation, e.g. those in the Sahel.

Invertebrates
Scorpions and insects, such as ground beetles, have been reported as predators but there is no evidence that they cause significant mortality.

Hymenoptera: Sphecidae
Some sphecid wasps are specialist predators using Acridoidea to provision their nests. In Africa the best known is *Sphex aegyptiacus* Lepeletier which has been observed to accompany swarms (Haskell, 1955).

Parasitoids

The nymphs and adults of Acridoidea are unusual in that they do not have hymenopterous parasitoids, but they do have an unusually rich assemblage of dipterous parasitoids which can cause substantial mortality.

Diptera: Nemestrinidae
Several genera of nemestrinids parasitize Acridoidea, the most important of these in the Old World is *Trichopsidea*. *T. costata* Loew has a remarkably wide distribution and is present throughout Africa and the Mediterranean Basin parasitizing a wide range of species, possibly because it is dispersed as larvae within parasitized hosts. The adults lay large numbers of minute eggs in holes in wood, irregularities in the ground and other similar sites. The tiny triungulin-like hatchling larvae seek and enter their acridoid hosts where they feed attached to a respiratory tube growing from the entry point. Death of the host usually follows the exit of the fully fed third instar larva which burrows into the ground. Pupation is delayed until triggered by rain, as with bombyliid egg predators. Although adults of some genera have fully formed mouthparts and are able to feed, those of *Trichopsidea* spp. are vestigial (Greathead, 1958).

Diptera: Sarcophagidae
A large number of species of sarcophagid flies has been reared from Acridoidea, but many of them are saprophages (e.g. *Sarcophaga* spp.) or facultative parasitoids of newly moulted or moribund individuals (e.g. *Wohlfahrtia* spp.) so records need careful evaluation. Most true parasitoids belong to the genus *Blaesoxipha*. These flies are larviparous and place their larvae on the host which is entered through an intersegmental membrane. The species are not host specific but the host range is determined by the behaviour of the ovipositing female; thus, of the two most common African species, *B. filipjevi* Rohdendorf is attracted to crawling hosts and so is reared from nymphs and adults of a wide range of species but *B. lineata* (Fallén) attacks flying hosts and so is usually only reared from adults.

The number of larvae developing in a host is limited only by host size. Fully fed larvae leave the host, which usually dies rapidly from desiccation, and pupate immediately in the ground. There is no diapause in tropical species (Greathead, 1966b). They are easily cultured by teasing larvae from the ruptured bodies of gravid females and placing them on the open stump of an amputated leg.

Diptera: Tachinidae
Several genera of Tachinidae have been reported as parasitoids of Acridoidea, those most frequently encountered are *Acemyia* and *Ceracia*. They appear to be rare in Africa but Chapman (1962) reported up to 13%

parasitism of female *Nomadacris septemfasciata* (Serville) by *Ceracia nomadacris* van Emden in its outbreak area, the Rukwa Valley in Tanzania. Like *Blaesoxipha* spp. the tachinids are not host specific but tend to have favoured hosts, suggesting that they too select hosts in particular situations. They are viviparous or lay macrotype eggs on the host. The larva penetrates the host and feeds attached to the cuticle by a breathing tube. More than one larva may develop in each host.

Tachinids are usually less abundant than sarcophagids and have seldom been reported as important mortality factors. In fact there are no records of substantial parasitism of the larger pest species and no authenticated records from the desert locust.

Diptera: Muscidae

Muscid parasitoids have not been reared in the tropics but *Acridomyia* spp. are important parasitoids in North America and Eurasia. They are gregarious with up to one hundred larvae developing in a single host (e.g. Rees, 1973).

Parasites

Nematodes

Mermithid worms are frequently reported and have been studied extensively in North America. Although high levels of parasitism have been recorded in favourable habitats, they require moisture for maturation and oviposition and so are rare except in humid environments where grasshoppers are not usually a serious problem. See Rees (1973) for references and a discussion.

Pathogens

A wide range of pathogens have been isolated from Acridoidea but there are few quantitative assessments indicating their importance as mortality factors. Only the more important ones will be discussed.

Protozoa

Acridoid digestive tracts are frequently infected with protozoan parasites, e.g. *Malamoeba locustae* (King & Taylor) (Henry, 1968) and Eugregarina (Canning, 1956), which are often a problem in laboratory cultures but these parasites seem to be of little significance in field populations.

A more serious infection is caused by *Nosema locustae* Canning, which was first isolated from an insectary colony of *Locusta migratoria* (Linnaeus) in the UK (Canning, 1953). It causes a debilitating disease which reduces the life-span and fecundity of infected insects. *N. locustae* is now known to be widespread in field populations of locusts and grasshoppers and has been the subject of intensive research as a potential biological control agent (Brooks, 1988).

Bacteria

Bacterial infections recorded to date have all been due to non-specific agents which are potential human pathogens and so have been discounted as possible control agents. The most notorious of these was the disease reputedly caused by '*Coccobacillus acridiorum* d'Herelle' and used in abortive field trials as a potential biological pesticide until it was found that it was in fact a strain of a coliform bacterium, *Aerobacter aerogenes* (Kruse) (Steinhaus, 1949). Another bacterial disease isolated from a laboratory colony of desert locusts, due to a nonchromogenic strain of *Serratia marcescens* Bizio, was investigated by Stevenson (1959) who carried out a field trial with a bran bait inoculated with the bacteria but with equivocal results.

Spore forming bacteria, most notably *Bacillus thuringiensis* Berliner, have not been isolated from Orthoptera. The low pH of the gut would prevent the toxic crystal of most known strains from dissolving (Prior & Greathead, 1989).

Viruses

There are few records of virus infections from Acridoidea and most of these have only been reported once. The best known are entomopox viruses which have now been isolated from specimens collected in North America, Africa and Arabia (see table of records in Streett & McGuire, 1990). These large DNA viruses resemble vertebrate pox viruses superficially and therefore have been treated with caution; however, they are now known to have no close biochemical similarity and do not infect vertebrates (Streett & McGuire, 1990). Isolates from North American grasshoppers have been obtained and are being studied as potential control agents. However, entomopox viruses are not highly virulent and debilitate, rather than kill, the host. Furthermore, being obligate intra-cellular parasites they cannot be cultured *in vitro*.

The only other naturally occurring virus of Acridoidea, of possible interest as a control agent, is the crystalline array virus (CAV) isolated from grasshoppers in the USA. However, CAV was found to resemble picornaviruses of vertebrates and so studies on it were abandoned.

Nuclear polyhedrosis viruses are not known from Acridoidea in nature but there is an interesting report of the oral transmission of *Spodoptera littoralis* NPV to both *Schistocerca gregaria* and *Locusta migratoria* which causes visible symptoms termed 'dark cheeks disease' (Bensimon *et al.*, 1987).

Fungi

Fungi are among the most frequently reported pathogens affecting Acridoidea and records have been tabulated by Prior & Greathead (1989).

The best known fungal infections of insects are those caused by *Entomophaga* (*Entomophthora*) spp., known as the 'summit disease' because dying insects climb to the tops of stems where they die clasping the stem. Thus the dead insects are conspicuous and this may account for the frequency of reports of this disease compared with others which do not induce this behaviour. Epidemics in locusts and grasshoppers, usually identified as *E. grylli* Fresenius, have been reported from all regions where pest species have been studied, e.g. Skaife (1925) in South Africa, Chapman & Page (1979) in Nigeria, Pickford & Riegert (1964) in Canada, Roffey (1968) in Thailand and Milner (1978) in Australia (see also table in Streett & McGuire, 1990). Accounts note that prolonged periods of rain or warm humid weather are necessary to initiate and sustain epidemics and that although some acridoid species may be decimated others escape or are hardly affected. This variability in susceptibility has led to the recognition of many strains and some are now described as separate species. These fungi are obligate parasites and so cannot, at present, be cultured *in vitro*, furthermore, the resting spores have so far proved impossible to germinate artificially (Carruthers *et al.*, 1991).

Epidemics caused by Deuteromycotina, usually by *Beauveria* spp. and *Metarhizium* spp., have also been reported under similar circumstances to those reported for *E. grylli*. For example, Balfour-Browne (1960) provides a detailed description of the almost complete destruction of a swarm of desert locusts by *M. anisopliae* (Metschnikoff) Sorokin in Eritrea (Ethiopia) during unusually humid conditions.

Strains of these fungi do not always exhibit host specificity in laboratory tests but there are marked differences in pathogenicity to a range of test insect species which do not necessarily conform with the taxonomic affinity of the insects. The biosystematics and ecology of these associations are poorly understood and are the subject of current research. These fungi can be cultured readily *in vitro* on simple media and are already being exploited as biopesticides for froghoppers (Cercopidae) and other pests (McCoy *et al.*, 1988).

Impact of natural enemies on locust and grasshopper populations

There have been few rigorous studies on the population dynamics of Acridoidea which attempted to measure the role of natural enemies in population regulation. Quantitative studies over several years on essentially

static populations in temperate regions include those of Richards & Waloff (1954) on grasshoppers in England; Dempster (1957) on the Moroccan locust (*Dociostaurus maroccanus* (Thunberg)) in Cyprus and Smith (1965) on grasshoppers in Ontario. These studies indicate that natural enemies can cause important mortality but present no conclusive evidence that natural enemies are able to prevent outbreaks.

Zonocerus variegatus is the only tropical grasshopper on which quantitative population studies have been made. Chapman & Page (1979) found a surprising absence of egg parasitiods and predators but substantial mortality due to *Entomophaga grylli*, *Blaesoxipha filipjevi* and mermithid nematodes. However, the key factor in population regulation appeared to be fungal infection following rain.

The investigations on the desert locust mentioned in the introduction, and summarized by Greathead (1966a), point to the importance of natural enemies in contributing to the collapse of plagues but question their ability to prevent them.

The most comprehensive quantitative studies have been those on *Chortoicetes terminifera* by Farrow (1977, 1982a, 1982b) on populations in experimental plots. He concludes that natural enemies are only able to attain high mortality when generations overlap or when the population remains static. Key factor analysis identified migration as the factor determining population size. Natural enemy impact decreased during upsurges and increased during population decline, hastening the collapse of outbreaks.

Farrow & Longstaff (1986) modelled information on population dynamics of four tropical locusts: *L. migratoria*, *S. gregaria*, *Nomadacris septemfasciata* and *C. terminifera*. They concluded that in each instance weather and emigration were the key factors and that emigration prevented populations exceeding the carrying capacity and stopping natural enemies exerting any regulating influence. This result confirms beliefs of the authors cited above that natural enemies have little influence in plague dynamics.

Thus, natural enemies may on occasion provide a useful constraint on the rate of population increase, although they cannot prevent plagues, but probably have their greatest impact on declining populations. As such they merit conservation.

Potential of locust and grasshopper natural enemies for biological control

Classical biological control with insect natural enemies has been attempted on a number of occasions (Prior & Greathead, 1989) but with no success, and only two of nine introductions resulted in establishment. The possibilities

for classical biological control of static grasshopper populations merit further consideration. Some insect natural enemies can be eliminated from consideration because they are not amenable to handling in captivity, e.g. the egg predators (Bombyliidae, Meloidae) and nemestrinid parasitoids. *Stomorhina lunata* can be reared but there are questions to be answered about its biology before it can be recommended: viz. where does it breed in the absence of gregarious hosts, why is it absent from West Africa when it is present in the rest of the continent? The muscoid parasitoids are easy to multiply but introductions into North America and Hawaii have failed, suggesting that more careful selection of species for introduction is necessary before embarking on an introduction programme. Finally the scelionid egg parasitoids appear to merit further consideration. They are easy to rear but more information is required about host selection before recommendations can be made. There is currently renewed interest in the USA where species from Australia are being imported for trial (Dysart, 1991).

Multiplication of insect natural enemies for augmentative release is not considered feasible because of the large numbers required, problems of timing production in relation to phenology in areas of uncertain rainfall and the logistics of delivery. Pathogens are more promising for this purpose and *Nosema locustae* has been commercialized as a bait (Brooks, 1988). Although some promising results have been reported on the North American prairies overall the results have been equivocal and trials in the tropics have failed. Entomopox viruses are currently being investigated in the USA but the problem of economic production in living tissue is likely to restrict their use severely in the near future.

For swarming populations and also static populations the most promising approach to biological control appears to be the development of pathogens as biological pesticides. Here for the reasons already given those fungi which can be cultured *in vitro* are considered the most promising and are the subject of the current programme.

Since natural enemies have been shown to have an impact on acridoid populations they merit conservation and other control measures which have an adverse impact on them should be avoided or actions should be taken to minimize the damage. In particular it is necessary to monitor the environmental impact of chemical pesticides since a recent pilot study (Everts, 1990) has shown that fenitrothion and chlorpyriphos, now being applied for locust and grasshopper control in Africa following the banning of dieldrin, have an adverse effect on non-target organisms, including entomophagous arthropods and predatory birds which are natural enemies of Orthoptera.

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Fungal agents for biocontrol

M.S. Goettel

Agriculture Canada Research Station, Lethbridge, Alberta.

Abstract

Fungi have much potential to play a key role in the microbial control of grasshoppers. Successful development of a microbial insecticide will require careful selection of the best species and isolates. A brief review of the attributes and limitations of fungi as potential microbial control agents and of the several species of fungi commonly associated with grasshoppers are presented. Unlike most other insect pathogens which must be ingested in order to invade their host, fungi generally invade via the external cuticle. As with other biological control agents, fungi are limited by an array of biotic and abiotic factors. Although more than 700 species of entomopathogenic fungi are known to occur world-wide, only a handful are known to affect grasshoppers; among the most common are *Beauveria bassiana* (Balsamo) Vuillemin, *Metarhizium anisopliae* (Metschnikoff) Sorokin and *Entomophaga grylli* (Fresenius) Batko. It is concluded that there is presently an urgent need for a concerted effort to search for and isolate fungal pathogens world-wide.

Introduction

The first step in the development of a microbial control agent is the identification of those pathogens which infect the pest in nature. Among the different types of pathogens affecting grasshoppers are fungi. Fungal diseases in insects are common and widespread and often decimate insect populations in spectacular epizootics. This phenomenon illustrates the promising potential of fungi and has resulted in a considerable research

effort in an attempt to exploit these organisms as insect control agents; however, much more research is required and many problems must be solved before these organisms can be utilized on a larger scale for insect control. Recent reviews on the development of entomopathogenic fungi as microbial control agents are those of Hall & Papierok (1982), Lisansky & Hall (1982), Ferron (1985), Wraight & Roberts (1987), McCoy *et al.* (1988), Samson *et al.* (1988) and McCoy (1990).

Pathogenesis

Unlike most other insect pathogens which must be ingested in order to invade their host, entomopathogenic fungi usually invade via the external cuticle (Charnley, 1984; St Leger, in press). The infective propagule, usually a spore, attaches itself onto the integument surface, germinates, and penetrates the host cuticle. In some instances, fungi may also enter the host via the digestive tract or spiracles. Once the fungus has entered the body cavity, it spreads and colonizes the host's haemocoel. Some species produce a toxin within the host, and in such cases, host death may be rapid (Roberts, 1981). In species or strains that do not produce a toxin, host death is usually delayed and is generally thought to be the result of depletion of nutrients. After host death, the fungus emerges from the cadaver and, in the presence of high relative humidity, produces more infective propagules on the surface.

A full understanding of the factors affecting the ability of a fungus to enter and kill its host is paramount in the development of fungi as control agents. Factors that interfere in any of the critical steps could render the pathogen less efficient if not useless. Only recently have we begun to unravel the complex interactions between the host integument and fungal pathogen (St Leger, in press). Factors affecting these interactions include moulting, host defence mechanisms, fungal strain, presence of nutrients on host integument, and humidity, to name a few. Further details are given in the paper by Charnley (these proceedings).

Host range, specificity and virulence

Fungi have one of the widest host ranges among entomopathogens and, consequently, have the potential for use against most pest insects. Reports on wide host ranges of entomopathogenic species, however, are based on fungal species as a whole whereas laboratory studies demonstrate that different isolates of the same species may have varying degrees of specificity;

isolates are generally more virulent to the host from which they were first isolated (Goettel *et al.*, 1990a). Furthermore, fungi may even be more specific under field conditions and especially during epizootics.

Enhancement of virulence in entomopathogenic fungi has been a particularly neglected area of research. It has been demonstrated that, in general, virulence increases following successive passage through a host and decreases following repeated subculturing on artificial media (Aizawa, 1971; Roberts & Yendol, 1971; Daoust & Roberts, 1982). Such changes were thought to be due to gradual selection of genotypes; however, recently it was demonstrated that levels of cuticle-degrading enzymes were higher in conidia from infected tobacco hornworm larvae than those harvested from artificial culture, indicating that environmental conditions in which spores develop can pre-adapt them for the pathogenic mode (St Leger *et al.*, 1991).

Host specificity and virulence optimization need further investigation from the point of view of the range of insects infected, the virulence towards the target host, and the biotic and abiotic factors responsible for the extension or restriction of that virulence. Such information would enhance the development or choice of strains with optimal potential as control agents for specific pests or pest complexes and with minimal adverse effects on non-target organisms.

Biotic and abiotic limitations

As with other biological control agents, fungi are limited by an array of biotic and abiotic factors. The former limitations are still poorly understood. They include microbial antagonists on the host integument, leaf surface or in the soil; host feeding behaviour, physiological condition and age; and fungal strain. Abiotic factors include inactivation by sunlight, desiccation, presence of insecticides or fungicides, and temperature and humidity thresholds for germination and growth. Traditionally, because of a germination requirement for free water or a relative humidity of over 90%, relative macrohumidity has been considered as the most serious constraint on the use of fungi for insect control. However, recent studies indicate that fungal infections can occur at relatively low macro-humidities (Marcandier & Khachatourians, 1987). Indications are that the micro-humidities at the surface of the host integument or on the foliage may be sufficient for spore germination and host penetration (St Leger, in press); fungal spores formulated in oil have been shown to kill locusts at ambient humidities of 35% R.H. (Prior *et al.*, these proceedings).

As our knowledge of the biology, pathogenesis, and epizootiology of entomopathogenic fungi increases, novel strategies are being applied to overcome some of the many limitations. Through strategies such as selection of strains or recombinant DNA technology and proper formulation, many limitations may be overcome. The recent transformation of *Metarhizium anisopliae* (Metschnikoff) Sorokin to benomyl resistance through the introduction of a benomyl resistance gene from a non-entomopathogenic fungus, *Aspergillus nidulans* (Eidam) Vuillemin is the first example of the use of DNA recombinant technology in an entomopathogenic fungus (Goettel *et al.*, 1990b).

Fungal pathogens of grasshoppers

More than 700 species of entomopathogenic fungi are known to occur world-wide, but only a handful are known to affect grasshoppers (Table 1; see also Prior & Greathead (1989) for a list of species recorded from Orthoptera). Furthermore, most records are based on single occurrences. There is presently a need for a world-wide concerted efffort to identify and isolate fungal pathogens of grasshoppers.

Some promising candidates

Beauveria bassiana (Balsamo) Vuillemin

This fungus has been developed recently as a control agent of pest insects. It is being used in the USSR for control of the Colorado potato beetle

Table 1. Host records of fungal pathogens in grasshoppers.

Class/Species	Relative abundance[1]	Geographical area
Hyphomycetes		
Aspergillus sp.	rare	Spain
Beauveria bassiana	frequent	Africa, Australia, Brazil, North America
Metarhizium anisopliae	frequent	Africa, Australia, South-east Asia, South America
Metarhizium flavoviride	rare[2]	Ecuador, Africa
Sorosporella sp.	rare	USA, Africa
Entomophthorales		
Entomophaga sp.	rare	Indonesia
Entomophaga grylli (species complex)	frequent	Africa, Australia, Europe, North America, South-east Asia

[1]Abundance based on numbers of host records from various sources.
[2]This assessment may need to be changed in the light of recent discoveries in Africa.

(*Leptinotarsa decemlineata* (Say) (Coleoptera: Chrysomelidae)) and in the People's Republic of China for control of the pine caterpillar (*Dendrolimus punctata* (Walker) (Lepidoptera: Lasiocampidae)). Other isolates are being investigated as potential control agents of other pest insects, including *L. decemlineata*, fire ants (*Solenopsis* spp. (Hymenoptera: Formicidae)), black vine weevils (*Otiorhynchus sulcatus* (Fabricius) (Coleoptera: Curculionidae), and citrus root weevils (*Diaprepes* spp. (Coleoptera: Curculionidae)) (McCoy, 1990). Although this fungus has a very wide host range, with over 700 insect species being susceptible, recent research indicates that certain strains of the fungus are much more host specific (Goettel *et al.*, 1990a).

There are several records of *B. bassiana* infecting grasshoppers (Table 1; Dresner, 1949; MacLeod, 1954; Humber & Soper, 1986; Li, 1987; Moore & Erlandson, 1988) and it has been documented as the cause of an epizootic in a swarm of red locusts (*Nomadacris septemfasciata* (Serville)) in South Africa (Schaefer 1936). Some recent experimental studies have also demonstrated its pathogenicity to grasshoppers under laboratory conditions (Marcandier & Khachatourians, 1987; Moore & Erlandson, 1988). Other recent research has shown that grasshoppers are highly susceptible to a strain of *B. bassiana* isolated from a dead grasshopper in Montana (Johnson *et al.*, 1988). Grasshoppers that were fed wheat leaves treated with spores produced from a Mycotech isolate died within 10 days. In subsequent laboratory dose-mortality tests, spores of *B. bassiana* sprayed in water or applied on lettuce disks were found to infect and kill grasshoppers of other species and ages at economically reasonable levels of application. Experiments in which third-instar grasshoppers were fed lettuce disks treated with droplets of spores indicated mortality of over 60% at one million spores per hopper (Goettel & D.L. Johnson, unpublished data).

Although *B. bassiana* may infect insects via the respiratory system (Clark *et al.*, 1968) and alimentary tract (Gabriel, 1959; Bao & Yendol, 1971; Broome *et al.*, 1976; Yanagita, 1987), penetration through the external integument is the most common route of invasion (Vey & Fargues, 1977; Pekrul & Grula, 1979; Atuahener & Doppelreiter, 1982; Allee *et al.*, 1990). The route of invasion in grasshoppers is unknown; however, histopathological studies are under way in Lethbridge to determine the mode and site of infection in grasshoppers. Such information may greatly aid in the selection of the most effective methods for host targeting, product formulation and field application.

The development of methods for the mass production of this strain by Mycotech Corporation, Butte, MT, (see Goettel & Roberts, these proceedings) has allowed preliminary field trials to be conducted against grasshoppers in Alberta, Montana, Saskatchewan (C. Bradley, M.A. Erlandson, Goettel and D.L. Johnson, unpublished data), and Mali (see Johnson *et al.*, these proceedings). These applications included treatment of caged grass and crop, and also treatment of whole fields, using bran bait and oil-based spray formulations. Economic analysis by Mycotech has indicated that, if successful, an application rate of 10^{13} conidia/ha can be competitive in price with chemicals currently used for grasshopper control in the United States.

Metarhizium anisopliae and *M. flavoviride* W. Gams & J. Rozsypal

M. anisopliae is presently used on a wide scale in Brazil to control the sugarcane spittle bug (*Mahanarva posticata* (Stål)) (Roberts *et al.*, 1991; Mendonca, these proceedings); *M. flavoviride* has been successfully field-tested against the rice brown planthopper (*Nilaparvata lugens* (Stål) in the Philippines (Rombach *et al.*, 1986). There are numerous records of *M. anisopliae* from grasshoppers world-wide (Table 1; Balfour-Browne, 1960; Veen, 1968a; Humber & Soper, 1986) and it has been documented as causing an epizootic in a swarm of desert locusts in Ethiopia (Balfour-Browne, 1960). *M. flavoviride* was isolated from a grasshopper in the Galapagos Islands (Evans & Samson, 1982). Although this fungus has not been observed causing an epizootic in grasshoppers, recent laboratory studies have demonstrated that it is highly virulent against locusts (see Prior *et al.*, these proceedings).

There have not been any field tests to evaluate the potential of *M. anisopliae* or *M. flavoviride* against grasshoppers. However, such tests are warranted. *M. anisopliae* can be cultured using the solid-state fermentation technology developed by Mycotech (C. Bradley, pers. comm; Goettel & Roberts, these proceedings), or by low-tech methods discussed by Mendonça (these proceedings). Laboratory studies have demonstrated that *M. anisopliae* conidia ingested by desert locusts are killed by antifungal toxins produced by the gut bacteria: axenic locusts which were starved post-inoculation succumbed to the disease (Dillon & Charnley, 1986). Therefore, an innovative strategy for formulation of *M. anisopliae* would be to incorporate both an antimicrobial and stomach poison which would allow the fungus to penetrate via the gut.

Entomophaga grylli (Fresenius) Batko complex

E. grylli is an obligate grasshopper pathogen responsible for numerous epizootics in grasshoppers world-wide (Table 1; Skaife, 1925; Pickford & Riegert, 1964; Roffey, 1968; Milner, 1978; Chapman & Page, 1979; Erlandson *et al.*, 1988). It is regarded as the key factor regulating many grasshopper populations, often reducing populations to below economic thresholds. For instance, an epizootic in the clear-winged grasshopper

(*Camnula pellucida* (Scudder)) in the 1960s resulted in an 80% reduction in the required insecticide control costs of previous years saving farmers $30 million (D.L. Johnson, pers. comm.). It is generally believed that such epizootics require relatively humid environments with frequent dew formation; however, epizootics sustained in very dry environments have been recorded (R.S. Soper, R.A. Humber & B. Martinell, unpublished data as cited by Soper *et al.*, 1983). Several years of data from grasshopper populations in isolated mountain valleys of Arizona suggest that a combination of host and pathogen density-dependent relationships, spatial dynamics, and varying environmental conditions regulate disease prevalence (R.S. Soper, unpublished data as cited by Carruthers & Soper, 1987). More research is needed to fully elucidate the epizootiology of this fungus. (See also Paräso *et al.*, these proceedings).

Grasshoppers succumbing to *E. grylli* climb to the top of vegetation and die in the late afternoon with their heads pointing upwards (Skaife, 1925; MacLeod, 1963). Cadavers remain on the vegetation until they are either blown away by the wind or are washed off by rain. Soon after death, if the humidity is very high, fungal mycelia emerge through the intersegmental membranes and gradually encompass the cadaver. This mycelium produces numerous conidiophores which give rise to conidia which are forcibly discharged, showering many of the grasshoppers in the area. Conidia germinate on the host cuticle and penetrate into the grasshopper. Some cadavers killed by the fungus produce resting spores which develop within the host body. These are long-lived and initiate the disease cycle in another season.

Although presently recognized as a single species, *E. grylli* is actually a complex of pathotypes or possible species. These pathotypes may be recognized by host specificity and isozyme patterns (Soper *et al.*, 1983; Ramoska *et al.*, 1988). The existence of such great genetic variability throughout this taxon offers the potential for its use for classical biological control; recent work by Carruthers *et al.* (1991) is aimed at the importation of a particularly virulent pathotype of *E. grylli* from Australia for release in the USA.

Although *E. grylli* can be grown *in vitro* as protoplasts (MacLeod *et al.*, 1980), it produces infective stages only *in vivo*. Therefore, until an *in vitro* method for spore production can be developed, use of this fungus as a microbial insecticide would require mass production *in vivo* (see Goettel & Roberts, these proceedings). It may be possible to use *E. grylli* in the inoculative approach in order to initiate epizootics.

Conclusions

Fungi have the potential to play a key role in the microbial control of grasshoppers. However, our understanding of the epizootiology of fungal diseases is rudimentary; much research is needed before we can successfully use a fungus such as *E. grylli* to cause epizootics in grasshopper populations. Furthermore, very few fungal isolates from grasshoppers are available. Successful development of a microbial insecticide will require careful selection of the best isolates. There is presently an urgent need for a concerted effort to search and isolate fungal pathogens from grasshoppers world-wide – and the joint IIBC/IITA/DFPV programme provides an excellent opportunity to do this.

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Preliminary studies on the ecology of *Zonocerus variegatus* in the Republic of Benin

A. Paraïso[1], C.J. Lomer[2], I. Godonou[1] & D. Kpindu[1]

[1]*International Institute of Tropical Agriculture, Benin:*
[2]*International Institute of Biological Control.*

Abstract

Zonocerus variegatus (Linnaeus) is a very prevalent grasshopper species in Benin and forms a major focus of present ecological studies on grasshoppers. Parasites found so far include mites (Trombidiidae), the calliphorid fly *Blaesoxipha filipjevi* (Rohdendorf) and mermithid nematodes. The nematodes also attacked *Eyprepocnemis plorans ornatipes* (Walker). Quantitative studies on *B. filipjevi* are reported. Fungal infestation by *Entomophaga grylli* (Fresenius) Batko on *Z. variegatus* was observed in four regions. Several isolates of *Beauveria bassiana* (Balsamo) Vuillemin have been obtained from *Z. variegatus*. Some preliminary observations on other species of grasshoppers have been made. *Z. variegatus*, *Oedaleus nigeriensis* Uvarov, *E. plorans ornatipes* and several species of locusts and grasshoppers are being bred for pathology studies.

Introduction

Locusts and grasshoppers pose a continuing threat to food production in several African countries. An average swarm of locusts contains, in each square kilometre, 20–50 million insects weighing 40–100 t which can consume 40–100 t of vegetation per day. During a plague of locusts a swarm may cover a total area of several thousand square kilometres (Courshee, 1990). However, locust plagues occur sporadically while grasshoppers are more consistent in their occurrence in several countries, including Benin.

Zonocerus variegatus (Linnaeus) is the most important grasshopper

pest in Benin. The most heavily infested areas, covering the four southern provinces, are about 150,000 ha (Anon., 1986); but the insect occurs throughout the country, particularly in riverine habitats.

Other grasshoppers affect crops in northern Benin along the banks of the Niger river (Coffi, these proceedings). To date, the only means of controlling these pests has been to use insecticides (e.g. propoxur and fenitrothion) which are toxic to non-target organisms. With an increasing awareness of environmental problems associated with the use of chemical insecticides, it is important to consider non-chemical alternatives.

Numerous efforts were made to control locusts and grasshoppers with microbial agents, during the end of 19th century and the first half of the 20th century (Streett & McGuire, 1990). Several pathogens have been isolated from grasshoppers by different authors; microsporidians (Canning, 1953; Henry, 1967, 1971); viruses (Henry & Jutila, 1966; Henry *et al.*, 1969); bacteria (Stevenson, 1959); fungi (Chapman & Page, 1979); and nematodes (Denner, 1976).

Ecological studies on *Z. variegatus* and its natural enemies were made in West Africa by Chapman & Page (1979); work by other authors has been reviewed by Chiffaud & Mestre (1990). No studies on the ecology of *Z. variegatus* in Benin have been reported previously. The present study concentrates on the effects of parasites and pathogens.

Other species of grasshoppers have been little studied in Benin, although Fishpool & Popov (1984) provided an annotated species list.

Materials and methods

Surveys took place every month in the four provinces of southern Benin; Atlantique, Mono, Zou and Ouemé. Densities of *Z. variegatus* and other grasshoppers were recorded per square meter (Lecoq & Mestre, 1988). Information on host vegetation and damage was recorded. Collected *Z. variegatus* and other grasshoppers were brought to the laboratory and placed in cages to check for mites, parasitoids, nematodes and pathogens (Oma *et al.*, 1990). Samples of grasshoppers were dissected to evaluate the parasitism by flies and nematodes (Taylor, 1964). Diseased and dead grasshoppers were dried and incubated in moist petri dishes to look for fungi; tissues from dead and moribund grasshoppers were examined microscopically for other pathogens. Where disease symptoms were evident in the field (e.g. of *Entomophaga grylli*), counts were made of the number of diseased insects/m^2 to obtain approximate estimates of percentage mortality.

Results

Maps of the areas infested by *Z. variegatus* at high (> 20/m^2) and medium density (5–20/m^2) are shown in Fig. 1. *Oedaleus nigeriensis* Uvarov was discovered in high densities of up to 5–7 adults/m^2 and 3–4 larvae/m^2 in some areas. *Eypreprocnemis plorans ornatipes* was the second most commonly encountered grasshopper in southern Benin after *Z. variegatus*, although it was never present at high densities.

In the Lama forest, Zou Province, the density of *Z. variegatus* was 275 larvae/m^2 in October before the start of the survey, but decreased in November (Fig. 2). After November the density increased again due to recruitment of newly hatched larvae. Parasitism by mites reached 30% by December and 55% in March. In March up to 45 mites were counted on one grasshopper. This period coincided with the oviposition period when most of the adult *Z. variegatus* are crawling on the soil. By 15 April, the next generation of newly hatched larvae was present. Parasitism by *Blaesoxipha filipjevi* (Rohdendorf) (Diptera: Calliphoridae) was first noticed at the end of January and reached 15% by February, and 50% by mid-April (Fig. 3). In the Lama region, fungal infection by *Entomophaga grylli* (Fresenius) Batko was not observed during our surveys. However, in Cové region, epizootics of *E. grylli* occurred and led to 90 to 95% mortality of the population of *Z. variegatus*. The density of the dead grasshoppers reached 10/m^2.

In Mono province, high densities of *Z. variegatus* occurred in Lalo, Aplahoue, Athieme and Djakotomey. In Aplahoue the density was 380 larvae/m^2 in November, this decreased to 40 in January, coinciding with an increase in parasitism by mites and flies (Fig. 3). No fungal infection was recorded, and parasitism by nematodes in this region was very low. We also observed very low level of parasitism by the mites which increased in January, and in March reached 15%. Parasitism by *B. filipjevi* also began in January with 10% and increased to 22% by March (Fig. 3).

The second region in which a high level of infestation of *Z. variegatus* by *E. grylli* was observed was Ouemé. Here the level of parasitism by *B. filipjevi* increased from 0% in December 1990 to 50% in March 1991 (Fig. 4). At the end of this generation the complex of parasites and pathogens decreased as the *Z. variegatus* density fell below 10/m^2. In this region, mites parasitized 10% of *Z. variegatus* in January, increasing to 20% by March. The fungus *E. grylli* killed up to 80% of the *Z. variegatus* population in Sakete region decreasing the population density to 7/m^2.

In Atlantique province, one of the most infested regions is Niaouli, where the population density was more than 275 per m^2 in November. The level of parasitism due to mites was 3% in November and rose to 30% in March. In this region *E. grylli* killed 95% of the population of

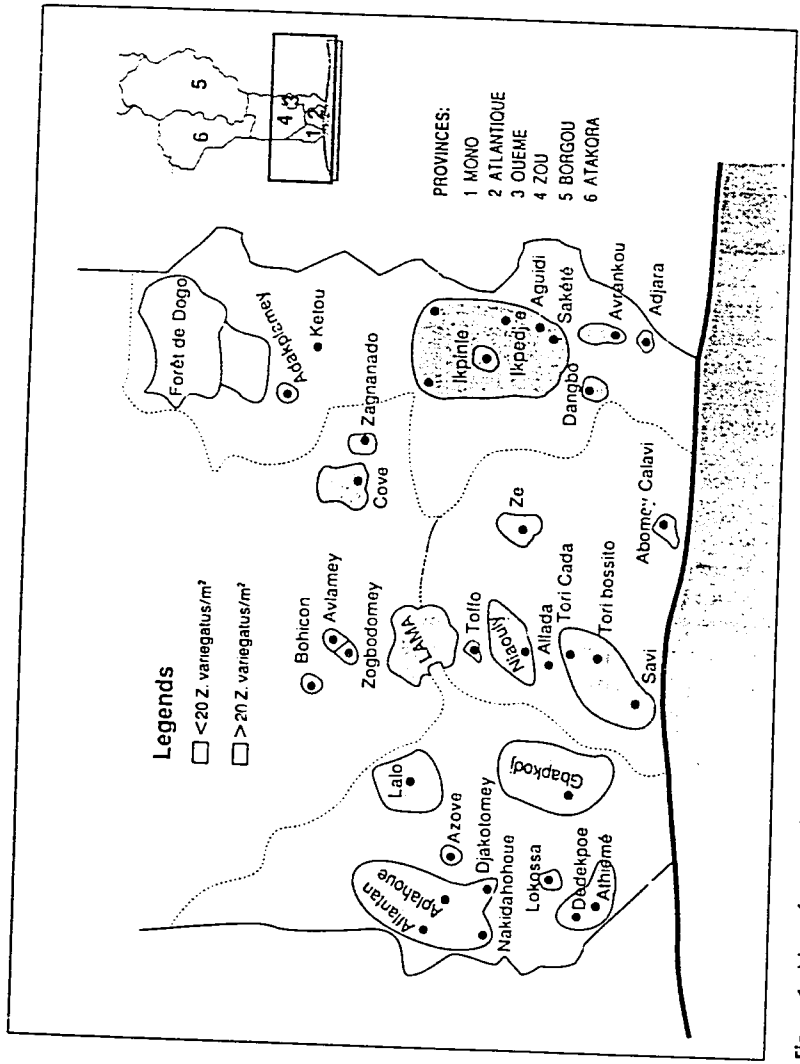

Figure 1. Map of areas infested by *Zonocerus variegatus* in southern part of Benin.

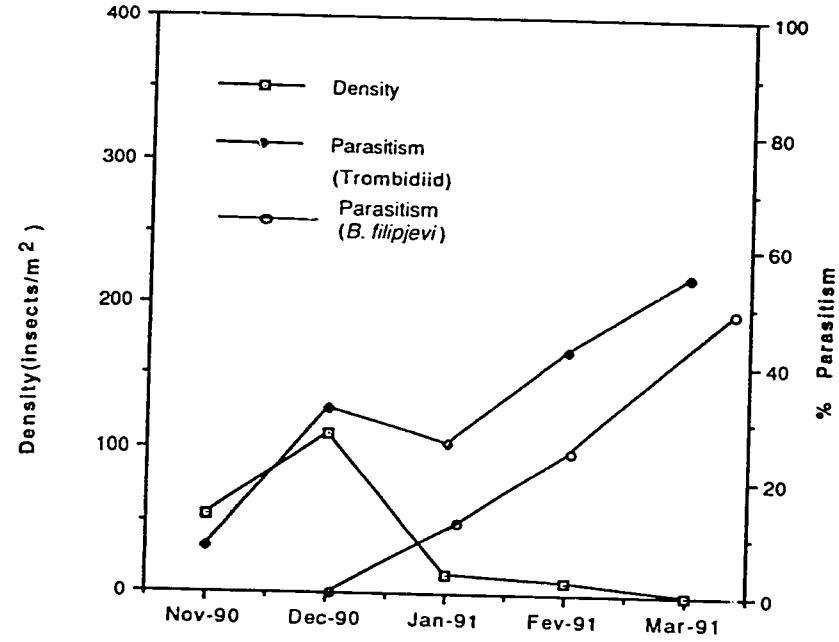

Figure 2. Density and level of parasitism of *Z. variegatus* in Lama, (ZOU Province).

Z. variegatus during February. Two isolates of *Beauveria bassiana* have been found in this region. The high density observed in November decreased in March to less than one *Z. variegatus*/m². Also in Atlantique, in Zê region, we observed an epizootic of *E. grylli* leading to the suppression of 90% of the *Z. variegatus* population; this was observed in both January and March. *B. filipjevi* parasitized 16% of the population in the same area in January and 60% by March (Fig. 5). In Zê, the level of parasitism due to mites was 30% in January. A third *B. bassiana* isolate was found about 50 km from Niaouli.

In all the regions we visited, oviposition sites of *Z. variegatus* were identified. Soil samples were taken from these different regions.

Pathogens

So far, three isolates of *Beauveria bassiana* (Balsamo) Vuillemin have been recovered from dead insects in the field; one was found on fifth instar larvae and the others on adults. The regions in which they were discovered

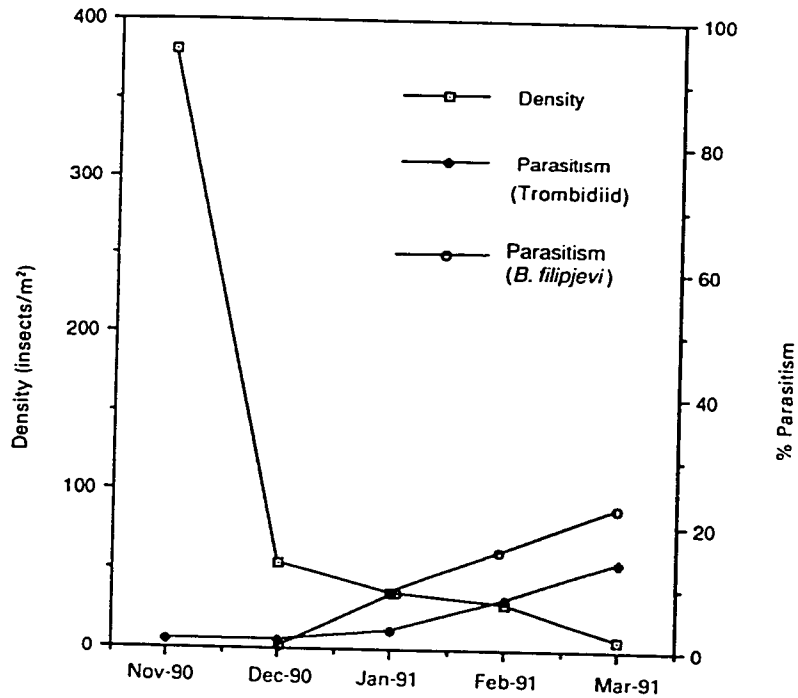

Figure 3. Density and level of parasitism of *Z. variegatus* in Aplahoué, (MONO Province).

are characterized by high levels of infestation of *E. grylli*. Unlike the *E. grylli* epizootics, which seemed to have a significant effect on the *Z. variegatus* populations, no epizootics of *B. bassiana* were observed in the field. We isolated *Fusarium* sp. from *Z. variegatus*, but it did not prove to be pathogenic on reinoculation. Laboratory tests have begun with the three isolates of *B. bassiana*.

Discussion

Oedaleus nigeriensis has not previously been reported in southern Benin. The zone in which it was found is characterized by the presence of young millet and wild Gramineae. However, severe damage by this grasshopper was not observed and the high density was very local.

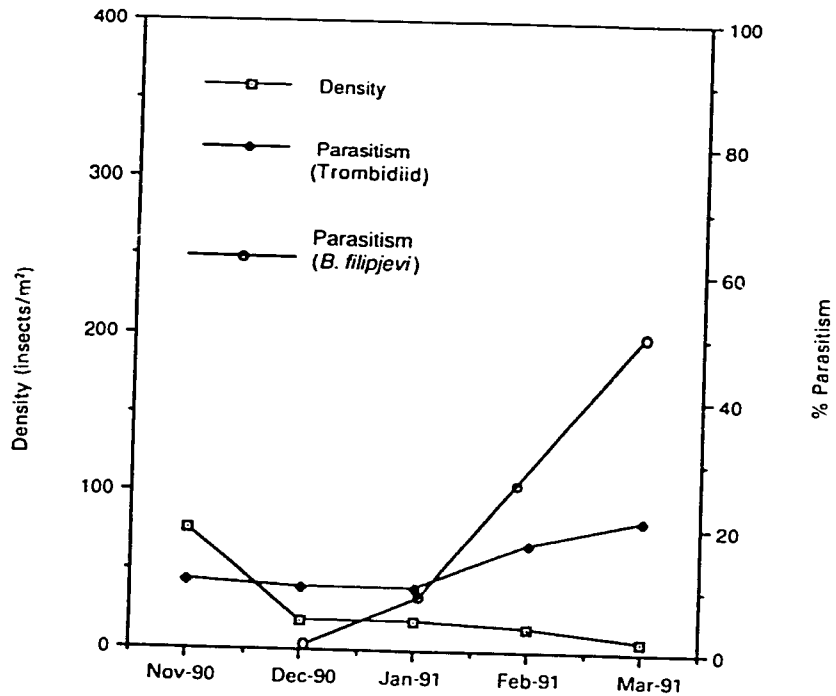

Figure 4. Density and level of parasitism of *Z. variegatus* in Avrankou, (OUEME Province).

Eyprepocnemis plorans ornatipes (Walker) is the second most often encountered grasshopper in southern Benin after *Z. variegatus*. Despite its density, little damage to crops was observed during current surveys in different localities in southern Benin.

A wide range of natural enemies is known to attack grasshoppers (see Greathead, these proceedings). Studies on *Z. variegatus* in Nigeria undertaken by Taylor (1964) and by Chapman & Page (1979) showed that *Blaesoxipha filipjevi* is the most prevalent parasitoid, with up to 60% of adults attacked. Similar levels were observed in the present study, e.g. 50% in Ouemé and 60% in Atlantique in March.

Chiffaud & Mestre (1990) in a review of the literature concluded that parasitized females would be unable to lay eggs and that levels of parasitism were sufficient to regulate *Z. variegatus* populations; however, critical studies to confirm a regulatory effect are lacking.

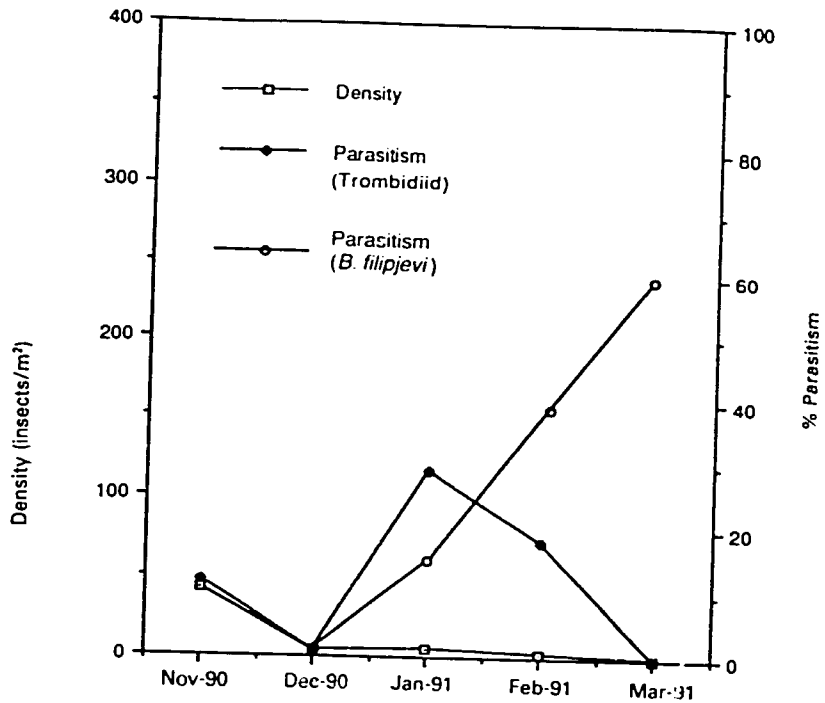

Figure 5. Density and level of parasitism of *Z. variegatus* in Zè, (Atlantique Province).

Studies on fungal pathogens showed that epizootics of *E. grylli* ofter led to 80% (Chapman & Page, 1979) or 99% infestation (Coulibaly *et al.* 1988). In Benin, epizootics of *E. grylli* were observed in four regions leading to the suppression of 80% to 90% of the population of *Z. variegatus*.

Our isolates of *Beauveria bassiana* from *Z. variegatus* are the first reported. Preliminary laboratory tests with the three isolates indicated good virulence in *Z. variegatus*, and will be the subject of our next publication.

The high level of parasitism by trombidiid mites seemed not to affect *Z. variegatus*, despite the high numbers occasionally observed on grasshoppers at the time of oviposition.

Parasitism by nematodes was very low, less than 1%, and in many places they did not appear at all. They seemed not to be specific to *Z. variegatus*.

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Effects of neem oil treatment on the phase status of desert locust, *Schistocerca gregaria*

J. Langewald[1] & H. Schmutterer[2]

[1]*International Institute of Tropical Agriculture:* [2]*Institut für Phytopathologie Universität Giessen.*

Abstract

Gregarious desert locust (*Schistocerca gregaria* Forskål) hoppers were treated with neem oil formulations containing different concentrations of azadirachtin in laboratory and field trials. Effects on the colour, morphology, and behaviour of the hoppers were examined.

First and second instar locusts were treated with neem oil at a concentration of 10 l/ha. Their aggregation behaviour was tested in small arenas after each moult following the treatment. The capability of the treated adults to aggregate, especially in larger groups, was significantly reduced compared to the untreated control.

For cage trials in the field, two neem oil formulations containing 0.2% and 0.4% azadirachtin, respectively, were each sprayed on 600 third instar hoppers which were then separated in four cages containing 150 hoppers each. An untreated control was also replicated in four cages. Each surviving locust was observed daily for damage to antennae and legs and for colour change. The results clearly show differences in the proportions of damage to antennae in the fifth instar and the adults. In the fourth instar, proportions of yellow hoppers were higher in the treatments compared with the control. In the fifth instar green coloured hoppers were found in higher proportions than in the controls. Positive correlations between the green (solitary phase) colour and antennae damage were observed in the fourth instar in the treatments and the control. In the fifth instar strong positive correlations could be found between the green colour and damaged antennae or legs in the treatments and the control.

Introduction

The neem tree (*Azadirachta indica*), indigenous to Burma and India, is now very common in Africa, especially in the sahelian region. The properties of this fast-growing tree are various. In India, neem products are commonly used for medicinal purposes and soap production. The timber is termite resistant and because of its drought resistance, grows well in arid zones. With regard to plant protection the secondary compound azadirachtin, a triterpenoid, is most interesting. Azadirachtin affects insects in different ways (Schmutterer, 1990).

Compared to other insect species, *Schistocerca gregaria* (Forskål) seems to be extremely sensitive to azadirachtin (Nicol, 1991).

Material and methods

Formulation

In the laboratory trials pure neem oil with an azadirachtin content less than 0.05% was used to avoid high mortality and therefore allow a range of sub-lethal effects to be studied. In the field, oil enriched with methanolic extracts from neem cake (residue of the oil pressing) was sprayed at azadirachtin concentrations of 0.2% and 0.4%.

Experiments

Behaviour trials

In the laboratory on a small scale, a design airbrush was used as a sprayer. 100 first and 100 second instar hoppers in the gregarious phase were placed in a 25 × 25 × 40 cm glass tray in the middle of one square meter of ground. The area was sprayed with one ml neem oil. After each moult the aggregation behaviour was tested. A method similar to Gillet (1973) was used in this trial. The groups 'treated as first instar' and 'treated as second instar' were studied separately. After each subsequent moult ten treated and ten untreated hoppers were placed in a round arena 30 cm (for the second to the fourth instar) or 45 cm in diameter (for the fifth and the adult instar). The observations were made under natural light conditions in a greenhouse at a temperature of 28°C. The locusts were crowded together and 30 min later when they had settled down, a photograph was taken and they were crowded again. Further pictures were taken in the same way after 60 and 90 min. The pictures were analysed in two ways. First, the aggregation behaviour in general was evaluated by counting

locusts sitting together at a distance, less than one body length. Secondly, their capability to aggregate in larger groups was evaluated by counting locusts sitting together in groups of more than three individuals. The counts for the three replicates (30, 60, 90 min) were added. Each trial was repeated ten times.

Semi-field trials

The aim of these trials was to analyse effects of neem oil treatments under small scale field conditions and to test the above mentioned enriched neem oil formulations. For this purpose small mosquito screen cages with 1.5×1.5 m ground surface and 1 m height were installed in a cassava field. Two cassava plants in each cage provided shelter. Because of the high cyanide content of the cassava these plants were not eaten. Two neem oil formulations containing 0.2% and 0.4% azadirachtin were tested on late second and third instar hoppers.

For oil application, a Micro-Ulva sprayer with a red restrictor loaded with five batteries, providing a flow rate of approximately 20 ml/min and a droplet size of 80–100 µm was used. For each treatment 600 third instar hoppers were sprayed in a 25 m^2 arena using a dosage of 10 l/ha. After the treatment, the hoppers were separated in groups of 150 individuals and placed in the cages. Four cages with untreated controls were also installed. Then the instar, colour (black-brown, green, yellow) and morphological damage (antennae, legs) to each surviving locust were recorded daily during a 33 day period until all locusts were adult.

Results

Behaviour trials

The results for both groups 'treated as first instar' and 'treated as second instar' were very similar. During the larval instars there were no significant differences in aggregation behaviour, while for the adults the number of locusts was significantly ($P < 0.05$, Wilcoxon Signed-Rank Test) lower in a distance closer than one body length (Figs 1 and 2) and the number of locusts in groups of more than three individuals was highly significantly lower ($P < 0.01$, Wilcoxon Signed-Rank Test) (Figs 3 and 4) than in the controls. The medians of the ten replicates are shown in Figs 1–4.

Semi-field trials

The first effects on treated locusts could be expected in the fourth instar, i.e., after the first moult following the treatment. Since adult locusts have

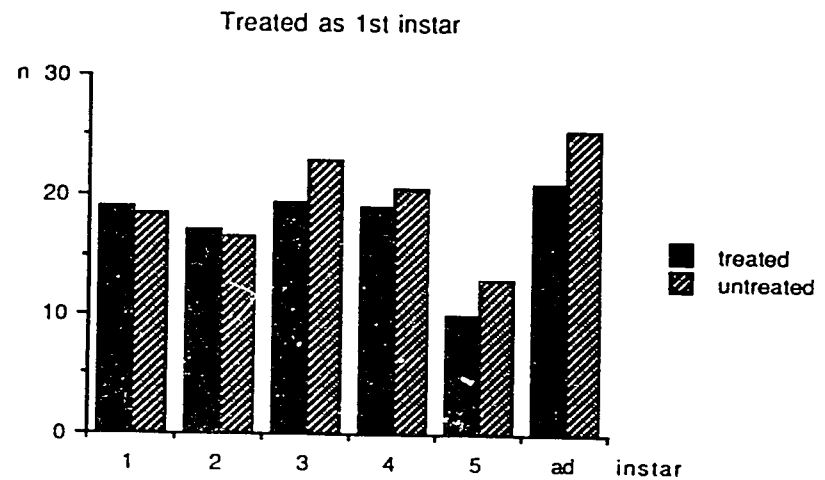

Figure 1. Number of grouped locusts in each instar.

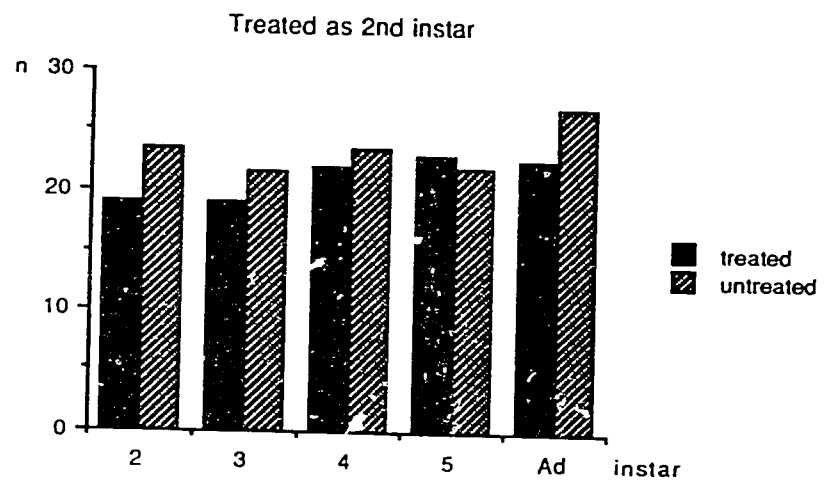

Figure 2. Number of grouped locusts in each instar after treatment.

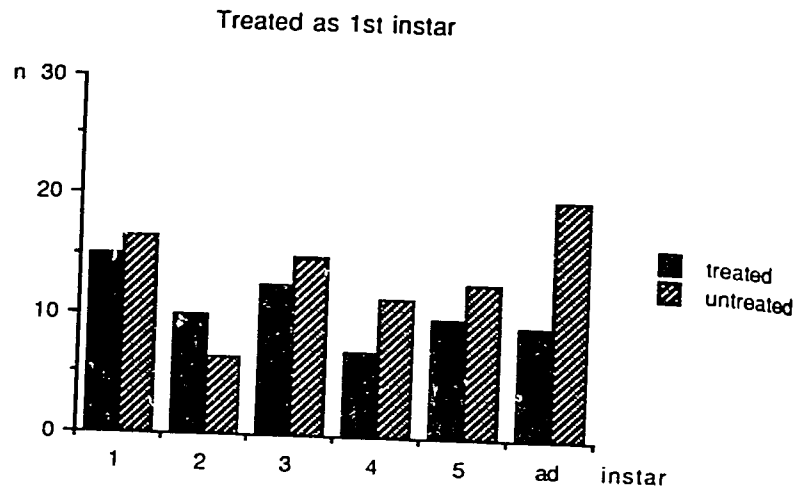

Figure 3. Number of locusts in groups composed of more than three specimens in each instar.

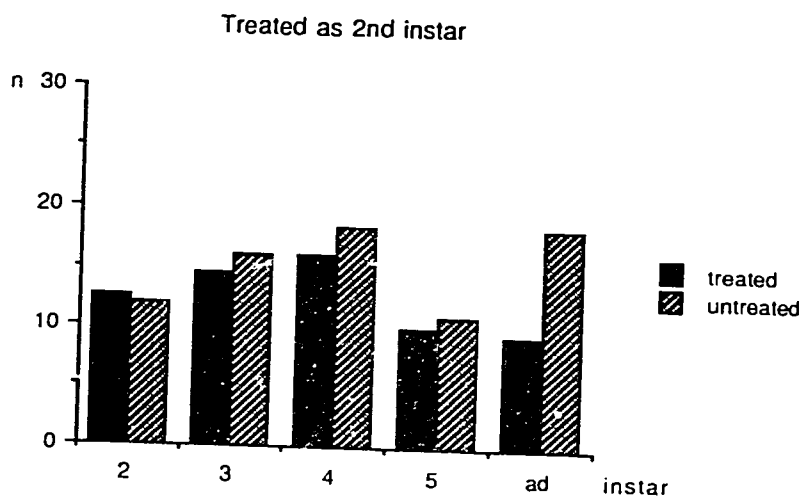

Figure 4. Number of locusts in groups composed of more than three specimens in each instar after treatment.

different colour characterisics from nymphs, the colours were not evaluated. For the presentation of results, the three day moving averages of proportions of damaged and differently coloured locusts are shown in Figs 5 to 8. To avoid misinterpretation, only those values from the period when >50% of the population was in that instar (indicated in each case by the dotted lines) should be examined. Outside of these values proportions could result from low numbers of locusts. The values of the replicates, for the days representing 50% of the population maximum were utilized in a one factor analysis of variance (Scheffe F-test and Dunnett t-test).

Morphological damage

Fourth instar
There was no significant difference between the treatments.

Fifth instar
On the 14th day the damage to antennae in the 0.4% treatment was significantly higher ($P < 0.05\%$) than in the 0.2% treatment and the control. The damage to antennae in the 0.2% treatment was also significantly higher than in the control of ($P < 0.05$).

On the 25th day the 0.4% treatment and the 0.2% treatment differed significantly from the control ($P < 0.05$) (Fig. 5).

There were no significant differences in the damage to legs.

Adults
On the 25th day damage to antennae was significantly higher in the 0.4% and 0.2% treatments compared to the control ($P < 0.05$) (Fig. 6). Damage to legs differed only significantly between the 0.4% treatment and the control on the 25th day in the fifth instar ($P < 0.05$) (Fig. 6).

Colours

Fourth instar
On the 14th day the proportion of yellow coloured locusts in the 0.2% treatment was significantly higher than in the control ($P < 0.05$) (Fig. 7).

Fifth instar
On the 14th day the proportion of yellow coloured locusts in the 0.4% treatment was significantly higher than in the control ($P < 0.05$) (Fig. 8).

On the 25th day the proportion of green coloured locusts in the 0.2% treatment and the control ($P < 0.05$), and the 0.4% treatment and the control differed significantly (Fig. 8).

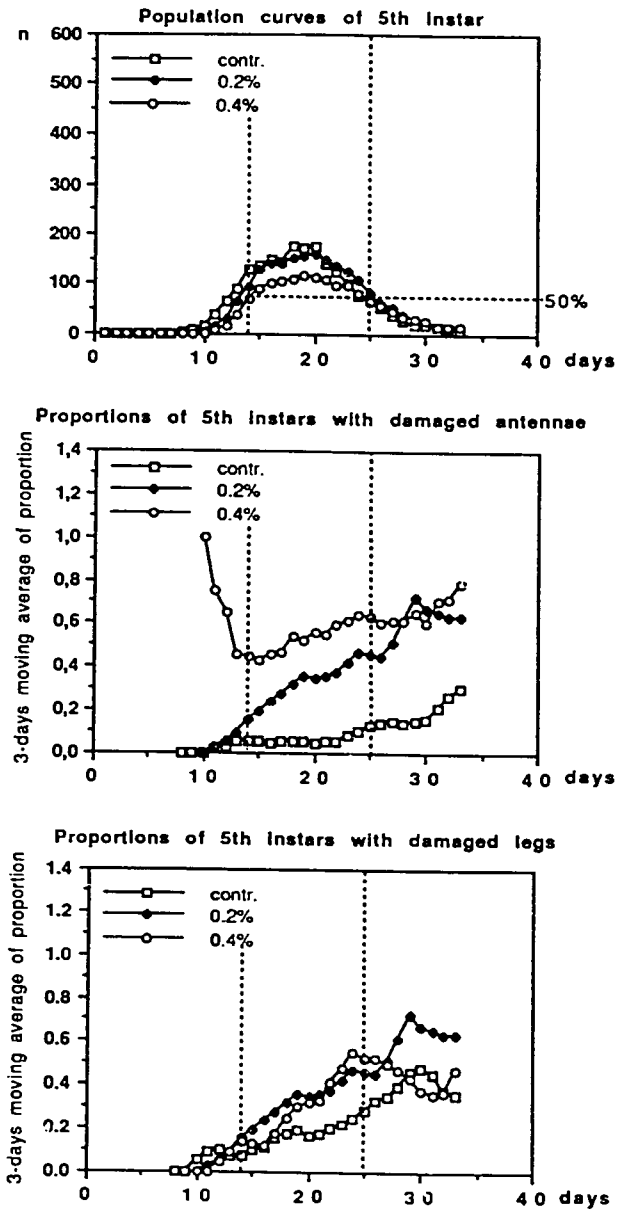

Figure 5. Proportions of damaged 5th instars during the experimentation period.

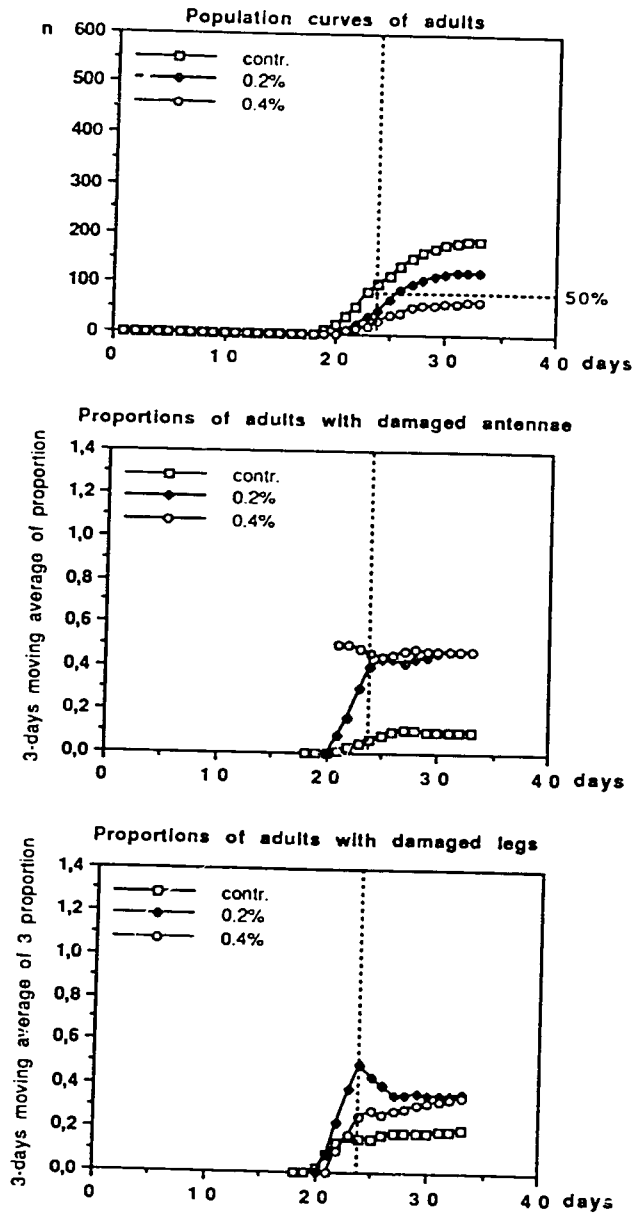

Figure 6. Proportions of damaged adults during the experimentation period.

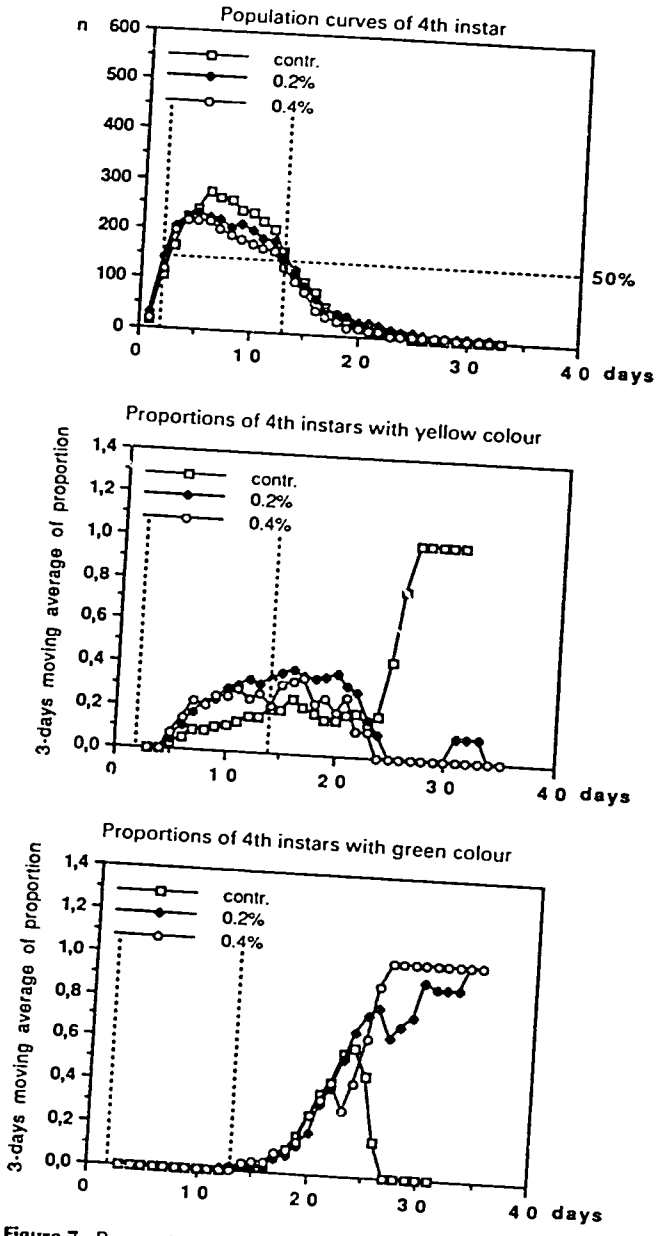

Figure 7. Proportions of 4th instars with yellow and green colour during the experimentation period.

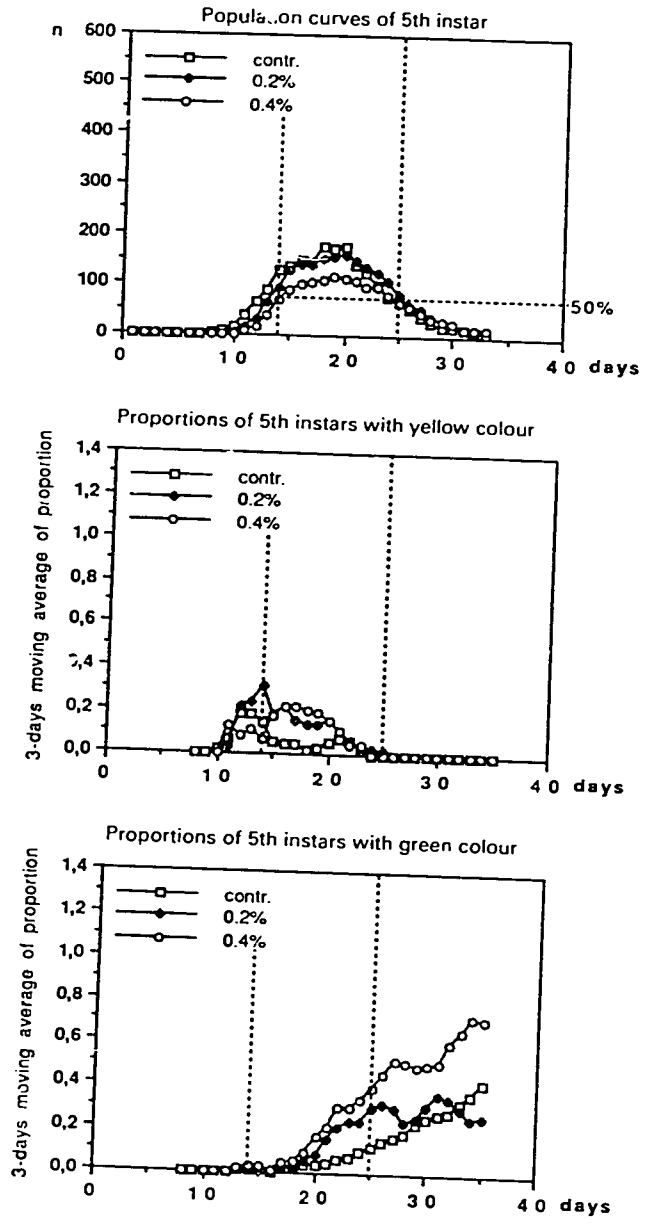

Figure 8. Proportions of 5th instars with yellow and green colour during the experimentation period.

Correlations

When the proportions of different coloured locusts are plotted against th proportions of locusts with damaged antennae and the proportions o locusts with damaged legs, in each treatment, clear correlations can be seer only for the fifth instar (see Table 1).

Fourth instar

In the fourth instar significant positive correlations between the proportions of green coloured locusts and the proportions of locusts with damaged antennae was found in the control (r=0.7; P=0.01) and the 0.4% (r=0.78; ($P < 0.01$) treatment.

Table 1. Correlations between proportions of different coloured locusts and proportions of locusts with damaged antennae or with damaged legs in each treatment, in the 5th instar.

		Correlations	
		r	P
Proportion damaged antennae			
Control	yellow	−0.55	0.04
	green	0.84	<0.01
	brown/black	−0.29	0.15
0.20%	yellow	−0.86	<0.01
	green	0.81	0.01
	brown/black	−0.19	0.47
0.40%	yellow	−0.71	0.01
	green	0.82	0.01
	brown/black	−0.52	0.13
Proportion damaged legs			
Control	yellow	−0.55	0.04
	green	0.84	<0.01
	brown/black	−0.29	0.15
0.20%	yellow	−0.86	<0.01
	green	0.81	0.01
	brown/black	−0.19	0.47
0.40%	yellow	−0.71	0.01
	green	0.82	0.01
	brown/black	−0.52	0.13

A significant correlation was found between the proportions of the yellow coloured and the proportions of the locusts with antennae damages in the control (r=0.7; P=0.02).

No significant correlation between leg damage and colour was observed.

Fifth instar

In the fifth instar the results were more obvious. It is clear that proportions of green locusts have a significant positive correlation, while the proportions of the yellow coloured locusts have a significant negative correlation, with the proportions of locusts with damaged antennae in all three treatments.

Exactly the same is true for the correlations between the proportions of green and yellow (except yellow in the control) coloured locusts and the proportions of locusts with damaged legs (see Table 1).

Discussion

The experiments show that damage to extremities, caused by neem, leads to a change of colour in locust hoppers towards green, the colour of solitary nymphs. It was observed that this solitarizing effect also influences the aggregation behaviour. It is known from the literature (Mordue, 1977) that locusts at a certain population density may produce a gregarization pheromone which may be perceived by the antennae. If the neem oil treatment causes damage to the antennae, the affected locusts are no longer able to receive this pheromonal input and show a tendency towards solitarization. Since the change of colours in locusts treated with neem oil is much more intensive than in locusts with amputated antennae, damage to antennae cannot be the only explanation for solitarization (Schmutterer & Freres, 1990). The decline of juvenile hormone titres in treated locusts may also contribute to this phenomenon (Rembold, 1986). For practical locust control, a solitarizing effect as found after neem oil application could play an important role.

Acknowledgements

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Panel discussion: precis

Mr Niassy asked about egg parasites, the possibilities of fungal epizootics in the desert and the cost of formulations of fungal pathogens. Dr Greathead replied that there was little possibility of manipulating egg parasitoids as control agents. Concerning epizootics in the desert, Dr Goettel mentioned the possibility of conditions of high humidity following rain. Dr Prior added that formulating fungal spores in oil would reduce the requirement for high humidity; the cost of additions to the formulation, such as stabilizers, would be minimal.

Mr Adam commented that in Togo, *Verronia* sp. was found to act as a trap plant for *Zonocerus variegatus*. Dr Paraïso replied that this had not been tried in Benin. Mr Saizonou (Benin) commented that parasitism of *Zonocerus variegatus* builds up towards the end of the season. It is not clear whether the parasitic fly *Blaesoxipha filipjevi* has an effect on the population or not.

Captain Kitenda asked about the time to kill, and the survival of microbial control agents in the field. Dr Henry replied that while fungi could cause mortality in a few days, agents such as *Nosema* took several weeks. Activity of the microbes is reduced after 4 to 6 days; *Nosema* spores can persist for one year in the field, but cause insignificant mortality by that time.

Section III

Exploration and Characterization

Discovery and characterization of fungal pathogens for locust and grasshopper control

C. Prior

International Institute of Biological Control.

Abstract

An effective biopesticide must be based on a pathogen isolate with high virulence to the target pest, an acceptable host range and proven safety. Isolates of *Metarhizium* spp. with virulence to the desert locust, *Schistocerca gregaria* (Forskål), have been obtained both from within its known distribution and also outside it. All isolates with high virulence have been obtained from Orthoptera, but not necessarily from acridids. However, in other studies highly virulent isolates have been obtained from insects taxonomically unrelated to the target. Although such 'new encounter' isolates may be found they may be difficult to use because of quarantine concerns about their release in a new environment and their impact on non-target organisms. All isolates used as biopesticides for pest control must be proven harmless to the environment, and this will require the use of biochemical and genetic 'fingerprinting' in field tests to distinguish them from indigenous isolates already present. Concern may also be expressed over the stability of isolate genotypes which survive after application. Instability in *Metarhizium* spp. may arise either from mutation or from recombination via the parasexual cycle, but evidence suggests this may be rare under field conditions and restricted by incompatibility mechanisms. The known facts concerning the nature, geographical range and stability of genotypes in *Metarhizium* spp. do not support restriction on the distribution or use of these fungi for pest control.

Introduction

Locusts, especially the desert locust, *Schistocerca gregaria* (Forskål), and grasshoppers, including *Oedaleus senegalensis* (Krauss), *Hieroglyphyus*

spp. and *Kraussaria angulifera* (Krauss), are major pests in the Sahel and during plague years the desert locust may also cause damage to the more humid zones of Africa and Asia (Brader, 1988; Steedman, 1990). Pyrgormorphids, especially *Zonocerus variegatus* (Linnaeus), may also be targets for control. Grasshopper control costs in 1986–87 amounted to US$62 million (Brader, 1988). International donor contributions to desert locust control during the last plague 1985–89 were US$275 million (Anon., 1990). The scale of the problem is immense and the volume of chemical pesticides used in control has caused great concern to donors and the afflicted countries (Anon., 1990).

The collaborative research programme for biological control of locusts and grasshoppers being carried out by IIBC, IITA and DFPV seeks to develop pathogens as biological pesticides which may be integrated into the existing control infrastructure and thereby lessen the dependence on chemical control. The pathogens which have the most potential are members of the entomopathogenic Deuteromycotina, because these organisms are readily produced on simple culture media, have a proven safety record and are formulable as biological pesticides suitable for spraying using controlled droplet application technology at ultra low volume (ULV) rates of 1–2 (< 5) l/ha (Prior & Greathead, 1989). They have been used in commercial pest control operations in USSR and Brazil for many years.

Species in the two Deuteromycete genera *Metarhizium* and *Beauveria* have been recorded from a very wide range of host arthropods and there are several records from Orthoptera (Prior & Greathead, 1989). Other entomopathogenic Deuteromycete genera which have been recorded occasionally from Orthoptera include *Paecilomyces* and *Verticillium*, but the former includes some species pathogenic to mammals, and the latter are slime-spored and difficult to formulate in non-aqueous diluents for application at ULV rates. The joint programme is therefore concentrating on *Metarhizium* spp., which have proved more pathogenic than *Beauveria* spp. in assays to date.

The range of target species within the Orthoptera is wide. Although locusts and grasshoppers are taxonomically related, there are substantial differences in their ecology which affect control strategies. There is much evidence from studies on other insect pest groups that isolates of *Metarhizium* spp. and *Beauveria* spp. show considerable host specificity and will even show differential virulence to taxonomically related target pests. Both these factors indicate a need for careful selection of isolates with high virulence to the specific target pest under field conditions. Since it is not clear from the currently available information whether the best isolate for controlling a particular pest will originate from that pest, or even from one related to it (Prior, 1990), it is possible that a desirable isolate may originate from a country or even a biogeographical zone different from the one

in which it must be used. If the chosen isolate is exotic to the country where it must be used, it will be prudent to obtain quarantine clearance prior to import (Greathead & Prior, 1990).

In many countries, pathogens are subject to requirements for registration before they can be used in the same way as pesticides. The requirements include both mammalian safety testing, and environmental safety. The latter involves assessment of the impact on non-target organisms and of environmental contamination. An essential part of any work to meet the registration requirements will be adequate techniques to monitor the impact of the fungus after use. Since the species of *Metarhizium* and *Beauveria* are ubiquitous, a test is required to distinguish the test genotype from those already present in the test ecosystem. This requires characterization of the isolate using methods other than classical morphology because morphological criteria are insufficient to separate genotypes at the sub-specific level.

There are thus three questions to be answered with respect to selection of the best isolates for development:

1. where is the best place to seek the required virulent isolate?
2. if it is exotic in the country where it must be used, how can it be characterized so that its environmental impact, if any, can be monitored?
3. if it is exotic what are the risks, if any, involved in its use?

This paper discusses these questions, both in a general context and in the specific context of the discovery, development and characterization of isolates of *Metarhizium* spp. and *Beauveria* spp. for locust and grasshopper control.

Discovery – where to search?

What characteristics?

In order to develop fungal pathogens for controlling insects it is necessary to select genotypes which combine the best possible characters for killing the target pest under field conditions. It is obvious that the very best can never be achieved because the number of genotypes available is effectively limitless. It is therefore necessary to decide how to sample them adequately with the limited resources that are usually available. The characteristics that are desirable in an entomopathogenic fungal genotype can be divided into good production features, such as high sporulation, optimum utilization of available substrate and genetic stability, and good field performance, which can be summarized as high virulence to the target coupled with suitable host range.

Good production characteristics can be determined adequately in the

laboratory. They have been reviewed frequently (McCoy *et al.*, 1988; Bartlett & Jaronski, 1988). A high rate of conidial production per unit of substrate is very important. Conidiation in submerged culture would be most advantageous and is known for a few isolates of *Hirsutella thompsonii* Fisher (van Winkelhoff & McCoy, 1984) and *Beauveria bassiana* (Balsamo) Vuillemin (Thomas *et al.*, 1987), but not *Metarhizium* spp. Stability in storage is important and *Metarhizium* spp., in particular, are prone to lose the ability to sporulate if maintained on rich media or if subcultured frequently: storage in liquid nitrogen is recommended to avoid these changes. The main requirement for selecting optimum production characters is a wide range of genetic diversity to choose from, but criteria for identifying these characters in the field are difficult to define.

Virulence under field conditions is determined by a complex of characteristics, not all of which can be quantified under laboratory conditions. The terms virulence and pathogenicity are often used interchangeably and it is worth noting that there are clearly defined differences, at least in plant pathology (Talboys *et al.*, 1973):

- **pathogenicity** is the ability to cause disease and is used as a qualitative description of a genus or species. For example, *M. anisopliae* (Metschnikoff) Sorokin is pathogenic to a wide range of arthropods
- **virulence** is a quantitative measure of the capacity of an individual pathogen genotype to cause infection. Thus one may have an avirulent isolate (genotype) of a pathogenic species with respect to an individual host: many of the isolates tested against *Schistocerca gregaria* in the current programme have had little or no virulence to that host, but are none the less pathogenic because they are virulent to other hosts

When selecting for virulence in isolates for mycopesticide development, not all determinants may be relevant. Adhesion may play an important role in the natural infection process. However, the mechanical requirement for adhesion is circumvented in a contact biopesticide formulation by applying the conidia directly to the cuticle in a diluent. Similarly, the ability to produce conidia under dry conditions would be advantageous in the field because this would extend the time period in which natural spread would occur. The use of the fungus as a biopesticide also circumvents this requirement, since the conidial inoculum is produced artificially.

In the current programme for locust and grasshopper control, considerable effort has been made to search for new isolates in the breeding grounds of the desert locust and the main grasshopper species. A major reason for this is the existence of evidence for differences in temperature optima among isolates. McCoy *et al.* (1988) noted that entomogenous fungi are mesophiles, with temperature optima in the 20–30°C range. However, Milner (1989) noted the existence of a high-temperature isolate

of *M. anisopliae* attacking termites from northern Australia which had an optimum of 30°C and could sporulate at 37°C. Rath *et al.* (1990) reported a 'cold-active' isolate DAT F-001 of *M. anisopliae* which is under development for control of the pasture pest *Adoryphorus couloni* (Burmeister) (Coleoptera: Scarabaeidae) in Tasmania. Thus genotypes discovered in hot dry climates are more likely to be adapted to those climates.

A very high temperature optimum would however be undesirable because the risk of mammalian infection would cause great concern. Most isolates of *Metarhizium* spp. and *Beauveria* spp. show reduced or no growth at 37°C. It is not clear whether the hot- and cold-tolerant isolates obtained by Milner and Rath are stable for the temperature tolerance characteristic. This has been examined in other fungi and Burnett (1983) discussed warm-environmental and cool-environmental 'races of *Helminthosporium maydis* (Nisikado), the cause of southern corn blight in the USA. These were not stable and adapted to the alternate regime if exposed for 6–7 generations. There is thus a distinction to be noted here between selecting an existing isolate with an ecological adaptation to the higher or lower end of the mesophile range (as in *H. maydis*) and an attempt to extend that range beyond its limits by culturing and selecting at very high temperatures. It is not clear if the latter is possible and Milner's high temperature isolate may represent the limits of what is available.

Isolates of *Metarhizium* spp. or *Beauveria* spp. with tolerance of low humidity do not seem to have been detected, but Drummond *et al.* (1987) detected ability to grow at unusually low humidities in an isolate of *Verticillium lecanii* (Zimmermann) Viégas from aphids, suggesting that this trait would be worth seeking in other entompathogens.

'New encounter' or 'old association'?

The successful use of natural enemies for biological control was pioneered by entomologists and initially concentrated on the use of parasitoids or phytophages from the centre of origin of an insect pest or weed to control the pest in an outbreak area from which the natural enemies were absent. This 'classical' approach has been extended recently to the use of pathogens, particularly rust fungi, for weed control but has very rarely been attempted with pathogens for insects – the baculovirus of *Oryctes* is an exception. In the 1980s there has been considerable debate about the relative merits of old association versus new encounter agents for classical biocontrol (Hokkanen & Pimentel, 1984; Waage, 1990). The old association agents are those that have co-evolved with the target. They thus tend to have a high specificity, but some have suggested that they also have a lower impact because they have reached an evolutionary equilibrium with the host. By contrast, new encounter agents may have a devastating impact

but by their nature cannot be specific, because they must occur on at least one other host, the one on which they existed before the new encounter host arrived. There are not yet sufficient data to decide which of these two alternatives will provide the 'best' pathogen for development as a mycopesticide, but there are important implications in seeking a new encounter isolate.

The current research programme is concentrating on searches in the major breeding grounds of the desert locust, on the assumption that the most likely place to find isolates which combine virulence with host specificity is where the host is most constantly present. In the case of the desert locust the difference between old and new encounter pathogens is obscured by the locust's migratory habit, and it is likely that isolates might be obtained from anywhere within the plague area where breeding occurs. However, the greatest likelihood of finding locust-adapted isolates must still be in the areas where locusts occur most frequently.

There are acridid species in all hot, dry climates similar to the environments where the desert locust occurs, and in the New World there are many other species of *Schistocerca*. Since the range of hosts to which isolates of *Metarhizium* show virulence is usually wider than a single species, and often wider than a genus, it would be predictable that isolates with virulence to *S. gregaria* would be found both among New World *Schistocerca* spp. and among related orthopterans. While it is too early to draw conclusions, it is notable that all isolates of *Metarhizium* spp. which have shown high virulence to *S. gregaria* during the current work have originated from Orthoptera. These are listed in Table 1. It is also notable that not all isolates from orthopterans are virulent: a *M. anisopliae* isolate from an acridid in Pakistan and a *B. bassiana* isolate from *Zonocerus variegatus* in Benin were not virulent to *S. gregaria* using the standard bioassay.

To date, no examples have been found of non-orthopteran isolates of *Beauveria* spp. or *Metarhizium* spp. with high virulence to *S. gregaria*. However, Prior (1990) noted many examples from the literature where this was the case for other target insects (Table 2). Of particular interest is the routine use at the University of Bath of *M. anisopliae* isolate ME1 for studies on infection processes in several insects including *S. gregaria*: this isolate originated from the pecan weevil *Curculio caryae* (Horn) (Coleoptera: Curculionidae) in the USA (Dillon & Charnley, 1986), neither a host nor a country ever encountered by *S. gregaria*. This and other virulent isolates from non-hosts will be included in the current assays.

It would appear that a search among isolates from the target host and its relatives will reliably yield examples with high levels of virulence. The detection of high virulence in ARSEF 324 from *Patanga guttulosa* (Walker) is an example of a new encounter, albeit from a related host. The possibility that isolates from non-related hosts such as pecan weevil may

Table 1. Isolates of *Metarhizium* spp. tested against *Schistocerca gregaria*.

Isolate	Species*	Host	Country	% Mortality 5d	% Mortality 6d
ARSEF 324	*M. a.*	*Patanga guttulosa* (Walker) (as *Austracris guttulosa*) (Orthoptera: Acrididae)	Australia	88	88
IMI 330189	*M.f.*	*Ornithacris cavroisi* (Finot) (Orthoptera: Acrididae)	Niger	76	90
IMI 330189**	*M. f.*	*Ornithacris cavroisi*	Niger	81	96
IMI 324673	*M. f.*	*Zonocerus elegans* (Linnaeus) (Orthoptera: Pyrgomorphidae)	Tanzania	56	60
IMI 324673**	*M. f.*	*Zonocerus elegans*	Tanzania	32	72
IMI 168777ii	*M. a.*	*Schistocerca gregaria* (Forskål) (Orthopera: Acrididae)	Ethiopia	48	56
IMI 152222	*M. a.*	*Myllocerus discolor* Boheman (Coleoptera: Curculionidae)	India	16	40
ARSEF 2023	*M. f.*	Unidentified acridid	Galapagos Is	8	40
IMI 298059	*M. a.*	*Scapanes australis* Boisduval (Coleoptera: Scarabaeidae)	Papua New Guinea		20
IMI 047038	*M. a.*	*Aeneolamia varia* (Fabricius) (as *A.* (*Tomaspis*) *saccharina* (Homoptera: Cercopidae)	Venezuela	0	4
IMI 298061	*M. a.*	*Brontispa longissima* (Gestro) (Coleoptera: Chrysomelidae)	Papua New Guinea	0	0
IIBC 190–574	*M. a.*	*Acrotylus humbertianus* Saussure (Orthoptera: Acrididae)	Pakistan	0	0

M. a. — *Metarhizium anisopliae* (Metschnikoff) Sorokin; *M. f.* — *M. flavoviride* W. Gams & J. Rozsypal.
**Single conidium isolates from original mass conidial cultures. ARSEF — Agricultural Research Service Entomopathogenic Fungus collection; IMI — International Mycological Institute;
IIBC — International Institute of Biological Control.

also be virulent indicates that the geographical and biological distribution of virulence is wider than the host and its near relatives.

There have been suggestions that virulence is associated with high levels of protease, particularly chymoelastase, produced by the appressorium during cuticle penetration and ME1 produces high levels of this enzyme (St Leger, 1990). This might mean that there are general virulence determinants as well as the currently unknown specific determinants of

Table 2. Some examples of virulence in isolates of *Metarhizium* spp. and *Beauveria* spp. from taxonomically, ecologically or geographically isolated hosts.*

Fungus	Target/location	Source/location of most pathogenic isolate
Beauveria bassiana (Balsamo) Vuillemin	*Hypothenemus hampei* (Ferrari) (Coleoptera: Scolytidae) **Colombia**	*Cossus cossus* (Linnaeus) (Lepidoptera: Cossidae) **Italy**
Beauveria bassiana	*Chalcodermus bimaculatus* Fiedler (Coleoptera: Curculionidae) **Brazil**	unidentified vespid (Hymenoptera: Vestpidae) **Brazil**
Beauveria bassiana	*Diuraphis noxia* (Mordvilko) (Homoptera: Aphididae) **USA** (introduced)	*Schizaphis graminum* (Rondani) (Homoptera: Aphididae) **USA** *'Boverin'* (unidentified source host) **USSR**
Metarhizium anisopliae (Metschnikoff) Sorokin	*Nasutitermes exitiosus* (Hill) (Isoptera: Termitidae) **Australia**	*Aeneolamia* sp. (Homoptera: Cercopidae) **Mexico**
M. anisopliae	*Diatraea saccharalis* (Fabricius) (Lepidoptera: Pyralidae) **Brazil**	*Zulia entreriana* (Berg) (Homoptera: Cercopidae) **Brazil**
M. anisopliae	*Culex pipiens* Linnaeus (Diptera: Culicidae)	many virulent isolates from Coleoptera, Lepidoptera and Homoptera hosts: none from target host
M. anisopliae	*Patanga guttulosa* (Walker) (as *Austracris guttulosa*) (Orthoptera: Acrididae) **Australia**	*Schistocerca gregaria* (Forskål) (Orthoptera: Acrididae) **Africa and Middle East**

*Examples drawn from published literature and the current IIBC/IITA/DFPV joint research programme. I am grateful to Dr J. Jimenez for permission to quote the unpublished example from *H. hampei*.

host range. There have been many attempts to correlate virulence with production of particular enzymes, especially those that assist cuticular penetration, and the results are well summarized by Heale *et al.* (1989): '... only a limited value can be assigned to any one cuticle-degrading enzyme trait amongst the many others which interact and summate in the expression of pathogenicity'. However, it would not be contradictory to claim that high production of an enzyme such as chymoelastase is a general virulence determinant (Charnley, these proceedings).

If this is eventually proved, a balance will then have to be struck between selecting strains which have good general pathogenicity determinants but are thereby relatively non host-specific, and strains which have very specific determinants and are thereby perhaps less virulent or attack too few hosts. At present, there is still insufficient evidence to use enzyme characteristics for isolate selection.

What is the true habitat of *Metarhizium* and *Beauveria* spp.?

Metarhizium spp. have been isolated from soil world-wide; *Beauveria* spp. have a similar very wide distribution, but are more frequently associated with insect remains (Domsch *et al.*, 1980). *B. bassiana* forms extensive rhizomorphs in unsterile soil in New Zealand (T.R. Glare, pers. comm.) and Germany (Keller & Zimmermann, 1989) and *M. anisopliae* does so in Australia (D. Holdom, pers. comm.); however, these would appear to be unusual cases. It is therefore not clear if these fungi genuinely flourish in soil, or whether they survive there in a dormant state awaiting a susceptible host.

Brady (1979) noted that *M. anisopliae* formed chlamydospores (asexual resting spores) but there are few references to their role in survival. The 'chlamydospores' of *M. anisopliae* from infected elaterid larvae illustrated by Zacharuk (1973) more closely resemble hyphal bodies (H.C. Evans, pers. comm.). Many experiments have been carried out on the survival of conidia in soil, and physical and chemical soil characteristics have a major influence. Conidia of *M. anisopliae* can survive at least two years in some soils (Keller & Zimmerman, 1989). *Metarhizium* spp. and *Beauveria* spp. can both be recovered from the soil either by direct plating on selective media or by baiting (Zimmermann, 1986). At present it is not clear whether what is being recovered are conidia, chlamydospores, mycelia surviving on fragments of chitin, or mycelia living on non-insect substrates.

Zimmermann (1986) used *Galleria mellonella* (Linnaeus) (Lepidoptera: Pyralidae) larvae to bait from moist soil. This insect is very susceptible to pathogens, probably because of its unusually sheltered normal habitat in beehives where fungal pathogens such as *Metarhizium* spp. do not develop. However, this may mean that isolates obtained on *G. mellonella* from soil have a low virulence to other insects. It may be more appropriate to use acridids to bait entomopathogenic fungi from soil. There are no published protocols for this technique, but it is under test at present.

There is no specific information available on the relative virulence of isolates of fungi such as *Metarhizium* spp. from soil and from insects. The great majority of isolates available and used in assays are from insects, but this mainly reflects the interests of collectors. A similar situation occurs with *Bacillus thuringiensis* Berliner: this too is widely distributed in the soil

but rare on insects, and many soil isolates are non-pathogenic. It may be that there are two diverse sets of selection pressures on the common entomopathogenic fungi such as *Metarhizium* spp. and *Beauveria* spp., one for optimum characteristics for soil survival and another for virulence to insects. If so, it is unlikely that the same characteristics will be optimum for both habitats.

Characterization – how much must be known?

The need for characterization at the sub-specific level

The search for superior genotypes for exploitation as biological pesticides will be a continuing process, but development can usually proceed meanwhile using the best that are available. As soon as a formulation is shown to be effective under laboratory conditions, field testing will be required. Field tests are essential to demonstrate efficacy, and also to show that the formulation is not harmful to the environment. In order to monitor the effect of a biopesticide treatment on the environment, methods must be available to detect and recover the pathogen after the treatment is applied. In the case of *Metarhizium* spp., this is complicated by the fact that, since *Metarhizium* spp. are distributed world-wide, the species will be native to the environment where the test is carried out and the test isolate must therefore be distinguished from the resident population before its effects can be clarified. There is therefore a need for methods which can distinguish genotypes at the sub-specific level. The safety and quarantine aspects of field testing of exotic isolates are dealt with in the following section.

The terms for defining variation in fungi below the species level are confusing, largely because some terms describe physiological or other phenotypic variants, and others describe the underlying differences in the genotype. Brasier & Rayner (1987) listed five disciplines, morphology, genetics, taxonomy, ecology and pathology, in which more than forty terms for variation within a species are used. The terms that commonly occur in insect pathology are variety, strain, isolate, race and pathotype.

Varieties are the only sub-specific groups that are distinguishable on morphological grounds and they may also have a distinctive host range. There are two varieties of *Metarhizium anisopliae*, *M. a.* var. *majus* (Johnston) Tulloch (conidia (9.0) 10.0–14.0 (18.0) µm long) which is confined mainly to scarabaeids, and *M. a.* var. *anisopliae* Tulloch (conidia (3.5) 5.0–8.0 (9.0) µm long with a wide host range (Tulloch, 1976). There is strong evidence that var. *majus* is diploid (Heale, 1988). *M. flavoviride* exists as *M. f.* var. *flavoviride* W. Gams & J. Rozsypal (conidia (7.0) 8.0–9.0 (11.0) µm long and *M. f.* var. *minus* Rombach, Humber & Roberts (conidia (4.0) 4.5–5.5 (6.5) µm long (Rombach *et al.*, 1986).

The term 'strain' is used by morphologists, geneticists, ecologists and pathologists, in each case with different implications (Brasier & Rayner, 1987). A strain is derived from an isolate and should be distinguishable from other strains on morphological, biochemical or pathological criteria.

Strain is a confusing term because it is defined in several ways based on different criteria for this separation and it has not been used in this paper. If attempts have been made to ensure genetic homogeneity of a culture by deriving it from a single uninucleate spore then it will normally be referred to as a strain, but in the context of insect pathology, at least for fungal pathogens, it seems safest to refer to individual fungal cultures as isolates and this is the only valid definition for initial isolations of mixed genotypes.

The feature of a pathogen that most interests insect pathologists is the possession of a unique genotype that controls virulence to a unique range of hosts. This is expressed phenotypically as a specific host range, and defines the pathotype. If there is a differential reaction between a pathotype and defined genotypes of a single host, the pathotype is the equivalent of a race in plant pathology. Unfortunately, to define a pathotype or a race rigorously requires sufficient host challenges to establish their uniqueness from all other pathotypes or races, and this is usually impractical. There is therefore much interest in the biochemical and physiological reactions of an isolate which might correlate with its host range, so that a pathotype might be defined without the need to carry out inoculations on a wide range of hosts.

In environmental studies it may be necessary to distinguish one isolate of a variety, such as *M. anisopliae* var. *anisopliae*, from other isolates of the same variety indigenous to the test area, and this cannot be done by morphology. In order to 'fingerprint' an isolate below the variety level, biochemical, physiological and genetic methods must be used.

Biochemical methods and DNA analysis for characterization

There have been several studies on variation within the species *Beauveria bassiana* using isoenzyme analysis and physiological tests, and the genus *Beauveria* was examined using similar tests to supplement differential morphological characteristics (Mugnai *et al.*, 1989). By comparing variation in isoenzyme profiles, reactions to the APIZYM test system and reactions to a wide range of nutritional and dye-based tests, a profile for each isolate can be built up. Esterases are one of the most useful isoenzymes for this purpose. Mugnai *et al.* (1989) found that *B. bassiana* was an extremely heterogeneous species but that more homogeneous groups of isolates occurred, including both those from one geographical area and those from one insect order. Bridge *et al.* (1990) examined 16 isolates of this species from a single host, the coffee berry borer *Hypothenemus*

hampei (Ferrari) (Coleoptera; Scolytidae), with a pan-tropical distribution and found that there was much less variation in characteristics such as esterase bands than in the species as a whole.

By contrast, a population of 23 isolates of *B. bassiana* from the mirid *Adelphocoris lineolatus* (Goeze) in northern Italy was much more variable with respect to esterase bands, and more variable than a population of 8 French isolates examined in the same study (Riba *et al.*, 1986b). Variability in α-esterases was also used by Poprawski *et al.* (1988) to show that isolates of *B. bassiana* from the weevils *Sitona* spp. (Coleoptera: Curculionidae) were much more heterogeneous in populations from southern France than from northern France and England. Since only esterase bands were compared in these studies, it is not known if the more esterase-variable populations were also more variable in other characteristics.

A study on 96 isolates of *M. anisopliae* var. *anisopliae* and var. *majus* by Riba *et al.* (1986a) showed very similar results to that by Mugnai *et al.* (1989) on *B. bassiana*. The var. *majus* isolates were homogeneous for isoenzyme profiles but the var. *anisopliae* isolates were very heterogeneous, with more homogeneous groupings of isolates from single geographical areas or related insect hosts.

Recently DNA analysis has been used to examine genotype variability in *M. anisopliae*; Milner (1990) has shown that three native isolates of *M. anisopliae* with virulence to larvae of three scarabaeid pests of sugarcane in Australia are genetically distinct and Pipe & Heale (1990) have developed an rDNA probe from *Saccharomyces cerevisiae* Meyer ex Hansen that can detect polymorphisms in nuclear DNA of *M. anisopliae*.

Evidence for and against sub-specific populations in *Metarhizium* and *Beauveria*

There is thus evidence that within the highly heterogeneous species *M. anisopliae* and *B. bassiana* there exist groups of distinct genotypes, indistinguishable morphologically, which have narrower host range and narrower dispersal than the species as a whole. This is borne out by a large number of studies on virulence of isolates, where it has been shown many times that individual isolates have a much narrower host range than the species as a whole. To give only one example, Samuels *et al.* (1989) tested a range of isolates of *Metarhizium anisopliae* against the rice delphacid *Nilaparvata lugens* (Stål) and found that the LT_{50} ranged from five to fourteen days and that isolates from *N. lugens* itself showed an LT_{50} range of 5–11 days.

However, as already mentioned above, there are many examples where a virulent isolate originated from a host other than the target, and sometimes in a geographical area where the target does not occur (Table 2). The

data of Samuels *et al.* (1989) also show that the issue of genetic adaptation is very complex, because the most virulent isolate from a host other than *N. lugens* was more virulent than the least virulent isolate from *N. lugens* itself. While the evidence certainly suggests that host-adapted genotypes and geographically distinct populations exist in *Metarhizium* spp. and *Beauveria* spp., it is also possible for new encounter genotypes to show a high degree of virulence (Prior, 1990).

Release – what are the risks?

Political and biological divisions within the environment

If the isolate used for field testing and ultimately for control is exotic to the country concerned, the quarantine authorities may wish to approve its use. It would clearly be ridiculous in biological terms to restrict the entry of an isolate of *M. anisopliae* into a country purely on the grounds that it originated in a neighbouring country if there are no ecological barriers between the two, because political boundaries defining countries are often of very recent origin and have no biological significance. The Sahel ecological zone, for example, extends unbroken across northern Africa and encompasses parts of Senegal, Mauritania, Mali, Burkina Faso, Niger, Chad, Sudan and Ethiopia. Desert locusts and many other organisms are distributed throughout this zone. When the isolate originates from outside the ecological zone in which release is contemplated however, quarantine requires more careful consideration.

The scientific basis for evaluation of the risks of release of exotic isolates

Prior (1990) discussed the scientific basis for regulating the release of naturally occurring (as opposed to recombinant) exotic isolates of entomopathogenic fungi, and noted that they posed three possible hazards: toxicity, allergenicity and direct infection of non-target organisms. The first two hazards are not unique to micro-organisms and are already covered by existing safety legislation, but infection of non-target organisms was a special issue because of the unique self-reproducing properties of biological as opposed to chemical pesticides. Prior (1990) noted that there were three questions to be answered concerning the potential hazard to non-target organisms:

1. Does the isolate show specificity at normal inoculum levels?
There is no information on the spore dose which insects receive during natural infection in the field. Under non-epizootic conditions insects would

usually be challenged by very low doses of conidia, but the dose may increase by several orders of magnitude during an epizootic in a dense insect population when conditions are suitable for sporulation. The concern is about specificity of an introduced isolate at normal inoculum levels; although the 'normal' level is difficult to define it must be assumed to be equivalent to the levels of native isolates. Therefore the issue is whether the introduced isolate poses a hazard not already posed by the native ones. Prior (1990) noted that the genes for pathogenicity (equivalent to virulence) are not closely associated with specific genes in the target pest, as is the case of gene-for-gene relationships in some plant pathogens, but seem to be widely distributed and may even occur outside the geographical or ecological range of the host itself. If this is so there cannot therefore be any additional risk in releasing what is already present. The exact extent of genotype range and diversity can only be determined by biochemical and DNA analysis on a comprehensive sample of isolates.

2. Is specificity retained at high levels of inoculum?
The concern here is that the natural specificity of the isolate may be lost when very high levels of inoculum are applied as a biopesticide for pest control. There is little evidence on this subject, but in one study on the field application of *Beauveria brongniartii* (Saccardo) Petch to control the European cockchafer *Melolontha melolontha* (Linnaeus) (Coleoptera: Scarabaeidae), Baltensweiler & Cerutti (1986) found no infection in non-target arthropods.

3. Is specificity a stable trait after release?
This is the most complex question. Stability implies that the isolate genotype will not change after release and therefore its host range and physiological requirements will remain constant. The principal fear is that if an exotic genotype did change after release it could thereby pose a threat to non-target organisms. There are only two ways that this could happen: mutation, or genetic recombination. The first can be dismissed very simply on two grounds: firstly, most mutations are deleterious and do not survive, and secondly there is no basis for assuming that a mutation is any more likely to arise in an introduced genotype than in the native genotypes which are vastly more abundant. However, the issue of recombination requires more careful examination.

Sexual reproduction has never been observed in *Metarhizium* spp. and *Beauveria* spp. and they are therefore classified in the Deuteromycotina. However, recombination without meiosis can occur in fungi through the parasexual cycle and this has been recorded several times for *M. anisopliae* (Heale, 1988) and recently for *B. bassiana* (Paccola-Meirelles & Azevedo, 1991). Thus the frequency with which parasexual recombination in these fungi may occur in nature is very important in determining their stability.

In the parasexual cycle, the monokaryotic mycelia of two different

genotypes fuse and the nuclei are exchanged so that the mycelium becomes dikaryotic. The dissimilar nuclei then fuse to give a diploid nucleus. Exchange of genes between the two genotypes occurs in the diploid nucleus to give chromosomes with new gene combinations. This is followed by a process of haploidization in which there is a progressive loss of chromosomes so that the haploid number is restored, but the genotype has changed (Heale, 1988; Clarkson, these proceedings).

In *M. anisopliae* (Riba *et al.*, 1985) parasexual recombinants were forced in the laboratory between var. *majus* and var. *anisopliae*, and between two auxotrophic mutants derived from a single isolate of var. *anisopliae* virulent to *Ostrinia nubilalis* (Hübner) (Lepidoptera: Pyralidae). The segregants from the cross between the two varieties were non-virulent; some of the segregants from the cross between the two mutants showed reduced virulence and some were as virulent as the parent.

These crosses made by Riba *et al.* (1985) were forced between two mutants derived from a single parent, that is, a single genotype. If a cross is to occur between two different genotypes then these must be compatible. There is in fact much evidence that incompatibility mechanisms exist in many fungi to prevent crossing. Some isolates of *B. bassiana* studied by Paccola-Meirelles & Azevedo (1991) would not form heterokaryons, possibly because of incompatibility mechanisms. These are widespread in several intensively studied fungi and one, *Aspergillus flavus* Link ex Fries, is of particular interest because it is both a plant and insect pathogen. Numerous vegetative incompatibility groups occur in *A. flavus* and these prevent the exchange of genes. In discussing this phenomenon, Wicklow (1990) notes that: 'Asexual genetic exchange under field conditions has not been reported for *A. flavus*, or any other fungus, but is probably limited to members of the same incompatibility group.'

At present there does not seem to be any information about the existence of incompatibility groups in *Metarhizium* spp., but it would be surprising if they did not occur.

Caten (1987) had previously used similar arguments to Wicklow's (1990) to suggest that variation in fungi is not as widespread or as important for survival and evolutionary success as previously thought, and the role of the parasexual cycle in nature may have been overemphasized. Evans (1988) argued that entomopathogenic fungi such as *Metarhizium* spp. had sacrificed their teleomorphs (sexual state), and the chance of variation by sexual recombination, for the advantage of rapid and massive asexual conidial production. They did this in order to exploit ephemeral energy sources, especially the periodic population explosions of insects occurring in disturbed habitats. While parasexuality would provide the major mechanism for instability in asexual fungi, there appear to be good reasons for thinking that it is unlikely to lead to new and undesirable genetic combinations under field conditions.

The apparent absence of the teleomorph in *Metarhizium* spp. and *Beauveria* spp. deserves comment. Teleomorphs have never been detected during more than a hundred years of study in a wide range of environments. However, new teleomorph-anamorph connections are continually being established, even in fungi that are even more familiar than the common entomopathogenic deuteromycetes. The discovery of *Didymella fabae* (Jellis & Punithalingam), the teleomorph of *Ascochyta fabae* Spegazzini, which causes leaf and stem blight of faba beans, was only announced this year (Jellis & Punithalingam, 1991): this is one of the most studied of temperate pathogens of a major crop. It is often the case that fungal teleomorphs are produced in the natural environment under the influence of specific environmental and nutritional conditions, and they may therefore be transient, or rare. Such conditions are not met in artificial culture which is where the great majority of studies are carried out. Despite the continuing absence of teleomorphs for *Metarhizium* spp. and *Beauveria* spp., the possibility of their existence should not be totally discounted. It should be noted that although the teleomorph of *B. bassiana* is not known, Booth (1961) described a *Beauveria* anamorph of the Pyrenomycete *Pseudeurotium bakeri* C. Booth; in the published description it differs from *B. bassiana* principally in having conidia which are twice as large (4 μm d.).

Discussion

The alternative options for seeking microbial control agents were summarized succinctly by Pusey (1990) in the context of microbial antagonists for plant disease control. The search should always begin with the resident antagonists because these will be adapted to survive in the environment where control must be achieved. However, micro-organisms from other environments may also have good potential activity, but may be prevented from operating because of environmental constraints. These constraints may be overcome by formulation.

In the search for the best isolates for locust and grasshopper control, it may also be the case that potentially valuable isolates occur outside the environment where control must be achieved. Thus, following Pusey's (1990) guidelines, a comprehensive search should seek virulent isolates from the target, other isolates from the target which lack virulence but which may be improved by formulation, and, in addition, isolates from other hosts and regions. This includes almost all isolates that might be found, but the possibility of overlooking isolates which could be improved by formulation may be excluded by carrying out the screening using the formulation itself. Even so, the range of potentially valuable material from target and non-target hosts that remains to be screened is enormous. The policy that is being followed in the current programme is therefore to screen as wide a range of isolates as possible, but with particular emphasis on two categories: those from orthopteran hosts and those from hot, dry environments to obtain isolates with tolerance for high temperatures.

A principle that is often advocated in the search for microbial antagonists for plant pathogens is to search in areas where the problem would be expected to be serious, but is not. This is particularly clear in the case of 'suppressive' or 'pernicious' soils where plant diseases, insect pests or nematodes do not develop. The cause of the suppression is often antagonistic micro-organisms which gradually increase to high population levels if the host organism is present over several years. Thus suppression occurs either in undisturbed soils such as pastures, or in fields where the same crop is repeatedly cultivated. A case of suppression of insects was recorded by Ko *et al.* (1982) where the 'perniciousness' of an Hawaiian soil to termites was associated with the presence of two entomopathogenic fungi. It is more difficult to identify examples of soils which might be pernicious to locusts and grasshoppers because of their high mobility. However, the soil is likely to be the primary source of inoculum for occasional epizootics of *Metarhizium* spp. such as that recorded on desert locusts by Balfour-Browne (1960) and the regular outbreaks of *Entomophaga grylli* (Fresenius) Batko on *Zonocerus variegatus* noted by Chapman & Page (1979). Screening of soils from habitats frequented by target species would therefore probably be worthwhile.

Characteristics that are desirable in the chosen isolates include high virulence expressed as rapid kill and/or cessation of feeding, thermotolerance, high sporulation capacity and compatibility with the chosen spray diluent. It would also be useful to search for additional characteristics such as tolerance to ultra-violet light and efficient substrate utilization. Many of these desired characteristics are automatically selected during the assays. These are carried out at low humidity, high temperature and in the chosen formulation, in order to reproduce closely the field environment, and the isolate selected for development has performed successfully in preliminary field trials.

Pusey (1990) noted that it may be unrealistic to search for a single 'magic bullet' isolate. The range of desirable characteristics is very wide and very unlikely to occur in a single isolate. There may be some advantage in combining several different virulent isolates in a single formulation, thus widening the range of virulence determinants available to challenge the host. Synergy between isolates would be advantageous both in increasing virulence and in broadening the host range when several pest species must be controlled.

Host specificity is clearly a complex issue. Adhesion may play a role but in the case of the current work on bioassays, the mechanical aspects of adhesion are at least partly overcome by applying the conidia directly in the

formulation, in which they adhere closely to the cuticle. None the less, the assays clearly demonstrate large differences in virulence, with the majority of isolates tested showing no virulence to *S. gregaria*.

The genotypes for virulence would in fact appear to be widely dispersed. It is also possible that virulence is controlled by two types of genes, those for host specificity, which restrict virulence to certain hosts, and those for general virulence which control the overall level of virulence within the whole host range. The protease *prl* gene in *M. anisopliae* currently under study in several laboratories may be an example of one such general virulence determinant. Evidence for the existence of genes for general virulence also comes from the studies by Riba *et al.* (1982) which showed that certain isolates of *B. bassiana* were more virulent to all 'races' of *Bombyx mori* (Linnaeus) (Lepidoptera: Bombycidae) than others; presumably these isolates may have had more active genes for general virulence. Probably only extensive DNA 'fingerprinting' studies on natural populations will clarify the distribution of genotypes.

The genetic control of virulence is not yet sufficiently understood to state whether the genes for virulence are universally distributed or whether they have a more limited geographical distribution. The widespread presence of new encounter isolates would suggest the former. The hazards that might arise from movement of isolates into new areas are already identified (Prior, 1990) and are discussed further here. It would appear from the relative ease with which new encounter virulence can be demonstrated, that genes for virulence to any specific host are already dispersed beyond the current range of that host. There does not therefore appear to be any scientific basis for restrictions on movement of genotypes from one biogeographical region to another. A satisfactory quarantine protocol for pathogen acceptance can only be based on the known biological facts and these do not suggest any reason for concern over the movement of isolates of *Metarhizium* spp. and *Beauveria* spp.

The use of pathogens for pest control could remove many of the major problems associated with chemical pesticide use, especially environmental pollution and toxicity to non-target organisms. Despite this, there exists a barrier to acceptance of pathogens that was defined by Freeman (1981) as 'pathophobia'. This irrational fear still exists and fuels ever-increasing demands for host range and safety tests (Evans & Ellison, 1990). The fear of pathogens is due largely to ignorance of their biology. Much remains to be done in convincing administrators and environmental pressure groups that the use of pathogens for pest control provides a safe alternative to chemicals. This can only be achieved by presenting a case based on an adequate basis of facts. The current research programme on locust and grasshopper control provides an excellent opportunity to gather those facts

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Mechanisms of fungal pathogenesis in insects with particular reference to locusts

A.K. Charnley

School of Biological Sciences, University of Bath.

Abstract
Control of appressorium formation may be related to host specificity among isolates of *Metarhizium anisopliae* (Metschnikoff) Sorokin. The cuticle-degrading endoprotease PR1 has an important role in host invasion. Variation in enzyme charge, and thus ability to bind to and degrade different insect cuticles, may influence isolate virulence. When fungal chitinase is slow to appear, cuticle-chitin may present a significant barrier to host penetration. Thus acylurea insecticides, which act by interfering with chitin synthesis in insects, can act synergistically with insect fungal pathogens. Antifungal phenols contribute to the gut barrier to infection in the desert locust and possibly other Orthoptera. Formulation of fungal conidia to reduce the effectiveness of the phenols may promote infection through the gut. The blood-borne cellular defences of an insect are often ineffective against virulent fungal pathogens. Secondary metabolites like the cyclic peptide destruxins produced by *M. anisopliae* may be responsible in part. Causes of death are probably complex and have not been defined adequately for any mycosis. Secondary metabolites, like destruxins, are clearly implicated in some cases. Timing and titre of production of such compounds may be amenable to selection and/or manipulation.

Introduction

It is clear from other papers in this volume that fungal pathogens of locusts and grasshoppers hold out particular promise as alternatives to chemical insecticides. Strain selection and improvement, based on a knowledge of the mechanisms of fungal pathogenesis, will be necessary to optimize the

killing power of such mycoinsecticides (see Clarkson, these proceedings). The physiological and biochemical interactions between locusts and their fungal pathogens have not been studied in detail. However, because of their large size desert locusts, *Schistocerca gregaria* (Forskål), have been used in a number of model studies on insect–fungus relationships. In this brief review I will bring together what we know about the processes of invasion, host response and cause of death for deuteromycete infections in insects and, where possible, highlight *Metarhizium anisopliae* (Metschnikoff) Sorokin/*S. gregaria* interactions.

Invasion

Fungi alone among insect pathogens actively invade through the exoskeleton (cuticle). In most, but not all, cases conidia of mycopathogens attach indiscriminately to host and nonhost cuticle (Boucias & Pendland, 1991). However, for successful invasion subsequent prepenetration behaviour must be adapted to the nature of the cuticle surface. For example cuticle hardness and nutrient availability appear to govern the differentiation of infection structures (appressoria) by *M. anisopliae*. St Leger *et al.* (in press) found that strains of *M. anisopliae* isolated from Homoptera, whose cuticle surface is contaminated with high levels of sugars derived from honey dew (excreta), produce appressoria under high nutrient regimes. Conversely Coleoptera-derived isolates were adapted to a low nutrient environment, such as probably pertains on the surface of the hard, thick cuticle of their hosts. Not enough acridid isolates have been examined in this regard, however, to draw any conclusions about adapted host recognition mechanisms.

Variations in pathogenicity can be a function of a pathogen's ability to penetrate host cuticle (Vey *et al.*, 1982). The chemical complexity and resilience of the outer region of the cuticle (the epicuticle) may make it of particular importance in deterring non-pathogenic fungi; it is not known how invading fungi breach the epicuticle. Ultrastructural evidence suggests that both enzymic hydrolysis and mechanical deformation are involved. Lipoprotein lipases secreted in culture by *Metarhizium anisopliae* could perhaps combine with endoprotease PR1 (see later) to aid penetration of the inner region of the epicuticle (polymerized lipoprotein) (Charnley, 1989).

The main body of the cuticle, the procuticle, comprises primarily chitin fibrils embedded in a protein matrix, together with lipids and quinones. The mechanical properties of the different cuticles depend on the proportions of the two main constituents, the nature and extent of hydration of the proteins, and the degree of sclerotization (cross-linking of the proteins

by quinones) (Hillerton, 1984). The last-named appears to have a particularly strong influence on penetrability. In the main, insects with heavily sclerotized body segments are invaded *via* intersegmental and arthrodial membranes or spiracles. Resistance to compressive force by sclerotized cuticle (exocuticle) suggests that it presents a stronger barrier than non-sclerotized endo- and mesocuticles. Since water extrusion accompanies sclerotization, water availability in addition to phenolic cross-links may be a factor limiting penetration in exocuticle. Although exocuticle from the desert locust supports growth and the production of cuticle-degrading enzyme activity by *M. anisopliae in vitro*, the cuticle remains intact (St Leger *et al.*, 1986a). Limited histolysis of the cuticle, therefore, during invasion *in vivo*, would provide sufficient nutrition to enable largely mechanical passage through the exocuticle. An ultrastructural study has shown that *M. anisopliae* can invade *via* segmental cuticle in the desert locust (E. Seyoum & Charnley, unpublished data).

Enzymatic degradation of procuticle is suggested by changes in the staining reactions of cuticles around penetrant hyphae. But in most cases absence of obvious zones of histolysis in regions where mechanical damage is reduced or non-existent suggests that fungal enzymes are active but limited to the vicinity of the fungal structure (Charnley, 1989).

In vitro studies on deuteromycete fungi grown on pulverized locust cuticle have revealed a range of extracellular cuticle-degrading enzymes that could potentially contribute to host penetration (St Leger *et al.*, 1986b). These enzymes correspond to the main components of cuticle, viz. protein, chitin and lipid. They appear sequentially. Esterase and proteolytic (endoprotease, aminopeptidase and carboxypeptidase) were produced first (< 24 h) followed by N-acetylglucosaminidase. Chitinase and lipase were produced 3–5 d later. Since chitinase from strain ME1 of *M. anisopliae* is an inducible enzyme and cuticular chitin is masked by protein, the late appearance of chitinase is presumably a result of induction, as chitin eventually becomes available after degradation of encasing cuticle proteins. The late detection of lipase appears to be due to the fact that the enzyme is largely cell-bound in young cultures.

Testing purified enzymes against locust cuticle *in vitro* showed that pretreatment or combined treatment with endoprotease (PR1, see later) was necessary for high chitinase activity. When locust exuviae (nondigested remains of old cuticle shed at ecydysis; exocuticle only) were used as substrate for purified pathogen enzymes instead of cuticle from larval sclerites (exo- and endocuticle), comparatively little hydrolysis occurred (St Leger *et al.*, 1986a). Similarly, while unsclerotized cuticle from fledgling locusts was rapidly degraded by fungal proteases, the crosslinking of cuticle proteins with gluteraldehyde (as a model for sclerotization) substantially reduced their susceptibility to proteolytic attack.

Extracellular endoprotease activity in culture filtrates of *M. anisopliae*

(strain ME1) grown on locust cuticle can be resolved into three main components, a chymoelastase (PR1), a trypsin (PR2) (St Leger *et al.*, 1987a) and a cysteine protease (PR4) (S. Cole, R.M. Cooper & Charnley, unpublished data). PR1 degrades locust endocuticle very efficiently; binding of this basic enzyme to negative charges on the cuticle is necessary for activity, and solubilized peptides are further degraded until a chain length of about 5 is obtained. In contrast to PR1, PR2 and PR4 have only minor cuticle degrading activity and may be involved with cellular control mechanisms, catalysing inactivation and activation processes.

Two classes of aminopeptidases have been isolated from locust-cuticle grown cultures of *M. anisopliae* and classified as an aminopeptidase of broad specificity, but with particular activity against alanyl residues, and a post proline dipeptidyl amino peptidase IV. Neither peptidase alone hydrolysed intact locust cuticle. However, when combined with PR1 they effected enhanced release of amino acids (R.J. St Leger, Charnley & R.M. Cooper, unpublished data). This is not surprising since the primary structure of locust cuticular proteins repeatedly contains the motif ala-ala-pro-ala/val (Hojrup *et al.*, 1986a). These exohydrolases presumably function *in situ* to break down PR1-derived peptides and thus provide nutrition for the fungus.

PR1 is produced constitutively, in the absence of repression by low molecular weight compounds containing carbon and nitrogen. Thus rapid synthesis of PR1 is only possible when the concentration of readily metabolizable compounds is low (St Leger *et al.*, 1988a). This is the case with locust cuticle as the components are largely insoluble until released by cuticle-degrading enzymes.

PR1 appears to be a pathogenicity determinant by virtue of its considerable ability to degrade cuticle (St Leger *et al.*, 1987a) and its production at high levels by the pathogen *in situ* during infection (St Leger *et al.*, 1987b; 1989; Goettel *et al.*, 1989). Simultaneous application of turkey egg white inhibitor and conidia significantly delayed mortality of *Manduca sexta* (Johannsen) (Lepidoptera: Sphingidae) larvae compared with larvae inoculated with conidia, supporting the importance of PR1 in penetration (St Leger *et al.*, 1988b). This kind of study has yet to be carried out with locusts.

Endochitinase and exochitinase (acetylglucosaminidase) have been purified from culture filtrates of *Metarhizium anisopliae* grown on chitin (St Leger *et al.*, 1991). However, there is no evidence for the production or activity of endochitinase during the first critical 40 h after inoculation of *Calliphora vomitoria* (Linnaeus) (Diptera: Calliphoridae) wings or abdominal body wall cuticle of *Manduca sexta*. The slow appearance of endochitinase *in vivo* is consistent with *in vitro* results (see earlier). It seems likely in *M. sexta–Metarhizium anisopliae* interactions that chitinase functions largely to provide nutrients during the saprophytic phase of

fungal growth in cuticle of moribund insect hosts (St Leger *et al.*, 1987b). This may not be so in locusts where the thick partially sclerotized body wall cuticle could delay penetration sufficiently to allow induction and activity of endochitinase.

The importance of chitin as a barrier to penetration and as a stabilizer of the cuticular protein matrix, in the absence of fungal chitinase, is evident from a study using the insecticide diflubenzuron (= Dimilin^R) a specific inhibitor of chitin synthesis in insects. Dual applications of diflubenzuron and *M. anisopliae* had a synergistic effect against larvae of *Manduca sexta*. Ultrastructural observations demonstrated that fungal penetration through diflubenzuron-treated cuticle was dramatically enhanced. Post-ecdysial diflubenzuron-treated cuticle (without chitin) was almost completely destroyed in contrast to pre-ecdysial cuticle (laid down prior to insecticide treatment) where hydrolysis was apparently selective (protein-only) and restricted to the vicinity of the fungal hyphae (Hassan & Charnley, 1989). Consistent with these ultrastructural observations, pharate fifth-instar *M. sexta* cuticle, produced during treatment with diflubenzuron and thus completely disrupted by the insecticide, was considerably more susceptible to Pr1 than the control cuticle. This study suggests that dual treatments of acylurea insecticides like diflubenzuron with entomopathogenic fungi could afford a useful strategy against locusts; particularly as second generation acylureas like teflubenzuron (= Nomoult^R) have more *in vivo* persistence and significant activity against the desert locust.

Lipase(s) are produced by *Metarhizium anisopliae* in culture on locust cuticle (St Leger *et al.*, 1986b). However, a true lipase (with activity against olive oil) was not extracted from cuticles of *Manduca sexta* and *C. vomitoria* infected with *Metarhizium anisopliae* (St Leger *et al.*, 1987b). In addition, potential substrates for a lipase, viz. triacylglycerols and wax esters, appear to be comparatively minor components at least of the 'extractable' lipids of locust as of other insect cuticle. Thus it is not clear what role, if any, lipase plays in cuticular penetration (Charnley & St Leger, 1991).

If virulence among fungal isolates were correlated (at least in part) with cuticle-degrading enzyme activity then an initial target for strain selection and improvement would have been identified. However, this link is difficult to prove. Comparisons between isolates for pathogenicity and production of enzymes may only reveal the great variability within a species for numerous factors, many of which may influence, but be unrelated to, cuticle-degrading enzyme activity. From what is known about the properties and occurrence of cuticle-degrading enzymes in fungal infections in insects at the present time, endoproteases appear to be particularly important to the penetration process. However, PR1- and PR2-like endoproteases are produced in quantity by all isolates of *M. anisopliae* and other deuteromycetes looked at (Charnley & St Leger, 1991). Cuticle-

degrading PR1 enzymes from *M. anisopliae*, *Beauveria bassiana* (Balsamo) Vuillemin, *Verticillium lecanii* (Zimmermann) Viégas, *Nomuraea rileyi* (Farlow) Samson and *Aschersonia aleyrodis* Webber differed only in charge. This has potential significance for their specificity as binding to cuticle is a prerequisite for activity. Cuticle from the migratory locust *Locusta migratoria* (Linnaeus) has a non-uniform distribution of charge, with negatively charged and positively charged groups predominating in flexible cuticle (e.g. arthrodial membranes) and rigid body-wall cuticle, respectively (Hojrup *et al.*, 1986b). What is known about the composition of desert locust cuticle suggests that it is similar to that from the migratory locust (Anderson, 1988). Thus it is possible that regions of the cuticle may be favourable or unfavourable to binding (and thus degradation) by individual enzymes, with consequences for the parts of the body which can be invaded by enzymatic action. This may influence speed of penetration and thus virulence.

Host defence

Insect alimentary canals are generally inhospitable to fungi. As a consequence, invasion rarely occurs from there despite the fact that both foregut and hindgut are lined with cuticle, which is largely unsclerotized and thus more susceptible than sclerite cuticle to pathogen enzymes. It is generally thought that anaerobiosis, digestive enzymes, adverse pH, speed of food throughput, and protection from the peritrophic membrane are primarily responsible for the gut barrier to fungi. In the case of the desert locust, however, antifungal phenols produced by the gut bacterial flora in the hindgut, make a significant contribution to host defence during periods of starvation when physical defences, such as gut movement and food throughput, prominent in fed insects, are less apparent. Fungitoxic phenols have also been found in the gut fluids of other related Orthoptera. The phenols are adsorbed by polyvinyl pyrolidone (PVP), thus formulation of conidia with PVP or other suitable material may reduce the effectiveness of the toxins and promote infection through the gut (Dillon & Charnley, 1991).

The appearance of host-produced brown or black pigments in insect cuticle in the vicinity of penetrant fungal hyphae is a common early sign of mycosis. Although the antimicrobial properties of oxidized phenols are well established, in insect cuticles melanization appears to be primarily a defence against weak or slow growing pathogens. Protease inhibitors within the cuticle may serve to restrict pathogen enzyme activity and hinder invasion (Charnley, 1989).

Once the cuticle and epidermis have been breached, the invading

fungus is faced with the defence systems of the haemolymph. In the desert locust the presence of *M. anisopliae* within the cuticle elicits an inflammatory response, some 12 h before the fungus enters the haemocoel, on the basement membrane of the epidermal cells beneath the infection site. The haemocytes also invade the basement membrane and enter the epidermis. Finally, melanization occurs, starting with the endocuticle close to the epidermis and developing backwards through the capsule. Despite this intense activity on the part of the host, the fungus is not prevented from invading the haemocoel (Gunnarsson, 1988).

Within the haemocoel the main defence against an invading fungus appears to be the entrapment of small elements of the fungus in a mass of sticky blood cells (haemocytes) (nodule formation) and the encapsulation of hyphal fragments by layers of blood cells. Central to these events is the recognition of non-self, and activation of the prophenoloxidase system (ppo) may be the key (Charnley, 1989). For *M. anisopliae* (strain RS549) in the desert locust, however, the cellular response to the fungus in the haemocoel is very poor and few blood cells adhere to penetrant hyphae (Gunnarsson, 1988). A similar situation has been reported for other insect–fungus combinations. Most entomopathogenic fungi are dimorphic, existing as yeast-like hyphal bodies in the host haemolymph during the pathogenic phase and converting to a mycelial form post-mortem prior to sporulation on the cadaver. The avoidance of host defence may relate to this change in growth form. Perhaps altered cell wall composition or the production of an antihaemocyte chemical, consequent upon this change in morphology, is responsible for the failure of the cellular defence. Certainly, evidence is consistent with interference of the immune system of the desert locust by the cyclic depsipeptide toxins, the destruxins (DTX), produced by *M. anisoplice* (Huxham *et al.*, 1989).

Endogenous protease inhibitors have been reported in insect haemolymph as well as cuticle (see above). These may be involved in blood borne defence against entomopathogens. Sixth instar *Anticarsia gemmatalis* (Hübner) (Lepidoptera: Noctuidae) larvae, which are resistant to infection by *N. rileyi*, contain high levels of serum protease inhibitor (Boucias & Pendland, 1987). Susceptibility of young *A. gemmatalis* larvae and larvae of all ages of *Trichoplusia ni* (Hübner) (Lepidoptera: Nocutidae) correlates with low titre or absence of protease inhibitor, respectively. Interestingly, haemolymph of the desert locust contains a potent serum protease inhibitor with activity against the cuticle-degrading protease PR1 (M. Hoppé & Charnley, unpublished data).

Host death

Death of an insect from an entomopathogenic fungus has been variously ascribed to toxicosis, starvation (either by competition between host and fungus for nutrients, or as a result of cessation of feeding by the insect), invasion of host tissues by the fungus, asphyxiating of the insect by fungal growth within the tracheae and/or interference with host physiology. However, it is impossible to ascribe a cause of death to a mycosis with any certainty. It seems likely that several factors will be involved. From the point of view of enhancing the killing power of a biocontrol agent, the involvement of a powerful toxin in host death is more amenable to selection and/or manipulation.

Destruxins may play a significant part in host death. Fifteen related compounds in the DTX series have been isolated from culture filtrates of *M. anisopliae*, although DTX A and B tend to predominate and DTX E is generally the most insecticidal. Low doses of DTX A cause tetanic and flaccid paralysis and death in Lepidoptera and adult Diptera, by causing depolarization of muscle membranes (Samuels *et al.*, 1988a). Evidence to date is consistent with DTX A causing depolarization by opening calcium channels in the membrane. Among strains of *M. anisopliae* pathogenic for *Manduca sexta* DTXs may act as virulence determinants by virtue of their effects on muscles. Strain ME1, which produces DTX *in vitro* and *in vivo*, grows sparsely in infected larvae and causes paralysis prior to host death. However, isolates RS549 and RS1094, which were less virulent than ME1 and took longer to kill *M. sexta* larvae, grew profusely in the haemolymph, did not induce paralysis in their hosts and produced considerably less DTX in culture than ME1. In addition, no DTX was found in the haemolymph of insects infected with RS549 and RS1094 (Samuels *et al.*, 1988b).

Understanding the role of DTX in pathogenesis is complicated by the restricted susceptibility to DTX of muscles from different insect orders in comparison with the wide host range of *Metarhizium anisopliae*. Only Lepidoptera and adult Diptera respond to low doses of DTX by paralysis and death (Samuels *et al.*, 1988b). As much as 100 μg/g insect DTX did not cause paralysis upon injection into adult desert locusts, and this low *in vivo* toxicity correlated with *in vitro* insensitivity of the muscles. However, DTX does interfere with function and cause ultrastructural changes in a number of tissues (particularly Malpighian tubules and haemocytes) in a variety of insects, including locusts. It is not yet clear whether the effects of DTX on different tissues may be attributed to a common mode of action. Nor is it clear how the various cytotoxic effects (other than on lepidopteran muscle) relate to symptoms and progress of disease caused by *M. anisopliae*. A two-tier system for the involvement of destruxin in pathogenesis has been proposed (Samuels *et al.*, 1988b). On the one hand in Lepidoptera and adult Diptera DTX may have a pathogenic role *via* action on the muscles. On the other hand it may have an aggressive role in those insects such as locusts whose muscles are not susceptible to DTX, and in Lepidoptera and adult Diptera infected with low DTX producing strains. In this case DTX may assist the pathogen to establish or extend its infection. In support of this, virulence of *M. anisopliae* isolates (as measured by LT_{50}) against the vine weevil *Otiorhynchus sulcatus* (Fabricius) (Coleoptera: Curculionidae) correlated with *in vitro* DTX production (E. Moorhouse, R. Kershaw, A. Gillespie, S.E. Reynolds & Charnley, unpublished data).

DTX is possibly just one of a number of secondary metabolites that *M. anisopliae* produces to promote its parasitic existence in insects. However, it seems likely that DTX has a significant role in pathogenesis.

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Molecular approaches to the study of entomopathogenic fungi

J.M. Clarkson

School of Biological Sciences, University of Bath.

Abstract

Many molecular techniques developed for other fungi are appropriate for use with entomopathogenic species. Genetic transformation of *Metarhizium anisopliae* (Metschnikoff) Sorokin has been achieved using cloned genes from *Neurospora crassa* Sheare & Dodge and *Aspergillus nidulans* (Eidam) Vuillemin. The *prI* gene from *M. anisopliae* which encodes a cuticle degrading protease, has been cloned recently. Improvement of strains of *Metarhizium* spp. and *Beauveria* spp. by traditional genetic methods has relied on exploitation of parasexual recombination since they lack a sexual cycle, but gene cloning methods now offer an alternative for improvement, if legislation will allow the use of laboratory produced recombinants. Molecular markers have been used to characterize the genotypes of individual fungal strains by examining gene products, but recent development of RFLP and PCR techniques allows direct examination of variability at the DNA level. Pulsed field gel electrophoresis (PFGE) is a technique which has been used to study karyotype variation in other fungi, and which could be used advantageously with entomopathogens.

Introduction

The recent development of molecular techniques for genetic engineering of filamentous fungi provides new opportunities for the study of entomopathogenic fungi. Cloning of genes encoding pathogenicity or virulence determinants will allow a rigorous testing of their role in insect pathogenesis and may provide the rationale for strain improvement by direct

genetic manipulation. The identification of molecular markers for characterizing fungal genomes makes it possible to study genetic variation directly at the DNA level. These new approaches are being applied to a wide range of fungi and will give new insights into the population structure of entomopathogens. The aim of this paper is to describe some of these molecular techniques and discuss how they might be applied to entomopathogenic fungi. Deuteromycete fungi, and in particular *Metarhizium* spp. and *Beaveria* spp., show the most promise as biological control agents of locusts and grasshoppers and will form the focus of this paper.

Molecular analysis of insect pathogenesis

An essential prerequisite for the molecular analysis of pathogenesis is the development of a method for the stable introduction of exogenous DNA. DNA-mediated transformation procedures for filamentous fungi are usually based on the addition of DNA to protoplasts in the presence of Ca^{++} followed by the addition of polyethylene glycol (Fincham, 1989; Timberlake & Marshall, 1989). Transformants usually arise at low frequency (0.1–10 transformants/μg vector DNA) through the stable incorporation of DNA into the fungal chromosomes, although there are now several reports of autonomous replication of transforming DNA (Tsukuda *et al.*, 1988; Powell & Kistler, 1990). Importantly, the identification of DNA sequences that promote autonomous replication also results in high frequency (100–10,000 transformants/μg vector DNA) transformation. A variety of selectable markers has been used for fungal transformation. Initially, transformation of the genetically well characterized 'laboratory' fungi *Aspergillus nidulans* (Eidam) Vuillemin (Ballance *et al.*, 1983) and *Neurospora crassa* Sheare & Dodge (Case *et al.*, 1979) utilized genes encoding catabolic or biosynthetic enzymes to complement appropriate mutants. Subsequently, dominant selectable markers such as hygromycin, bleomycin or benomyl resistance have been used, as these can be used to transform wild type strains of a genetically uncharacterized fungus.

Bernier *et al.* (1989) demonstrated recently that *Metarhizium anisopliae* (Metschnikoff) Sorokin could be transformed to benomyl resistance using a cloned β-tubulin gene from a benomyl resistant strain of *N. crassa* (Vollmer & Yanofsky, 1986). Transformants were stable under non-selective conditions and retained pathogenicity to *Manduca sexta* (Johannson) (Lepidoptera: Sphingidae). Southern hybridization indicated that the majority of transformants arose through the random integration into the chromosomes of tandemly repeated vector sequences. Benomyl resistant transformants of *Metarhizium anisopliae* have also been selected

by Goettel *et al.*, (1990) using a plasmid containing the *benA3* allele from *A. nidulans*. In both cases transformants were identified at low frequency. In *Beauveria bassiana* (Balsamo) Vuillemin resistance to chlorate has been used as a positive selection for mutants lacking nitrate reductase activity. The nitrate reductase (nia D) gene of *A. nidulans* was then used to transform a nitrate reductase mutant (Daboussi *et al.*, 1989).

Although in filamentous fungi, transformants arise mainly through the random integration of DNA, homologous integration can occur in a proportion of the transforming events (Fincham, 1989). This has been exploited to produce directed mutations by gene disruption or replacement with altered copies of cloned genes, thereby allowing a definite analysis of the role of that gene product. This strategy has been used recently to demonstrate that the cell wall-degrading enzyme polygalacturonase is not required for pathogenicity of the fungus *Cochliobolus carbonum* Nelson on its host plant maize (*Zea mays*) (Scott-Craig *et al.*, 1990). This powerful technique could also be used to investigate the role of putative pathogenicity or virulence determinants of entomopathogenic fungi.

Two areas that are already receiving attention are the production of cuticle degrading enzymes and insecticidal toxins (Charnley, these proceedings). Infection of the insect host occurs by direct penetration of the cuticle and *M. anisopliae* produces a range of enzymes capable of degrading components of the cuticle. Prominent amongst these is a protease (PR1) with chymoelastase specificity (St Leger *et al.*, 1987a). Several lines of evidence strongly implicate this enzyme as a determinant of pathogenicity. PR1 is the first of a sequence of cuticle degrading enzymes produced *in vitro* on host cuticle and is capable of extensively degrading cuticular protein (St Leger *et al.*, 1986, 1987a). It is produced in high levels by the fungus during host penetration (St Leger *et al.*, 1987b) and specific inhibition of PR1 delays disease symptoms and mortality on *Manduca sexta* (St Leger *et al.*, 1988). We have recently cloned the *pr1* gene (I. Paterson & Clarkson, unpublished data) and the production of a specific Pr1 deficient mutant of *Metarhizium anisopliae* by recombinant DNA technology should provide a definitive answer to the role of this enzyme in pathogenesis. Work on cloning the *pr1* gene is also in progress at the Boyce Thompson Institute, USA.

Insecticidal cyclic peptide toxins, destruxins, produced by *M. anisopliae* may play an important role in pathogenesis (Charnley, 1989). It is likely that destruxins are assembled extraribosomally using the thiotemplate mechanism now well established for the majority of other cyclic peptide antibiotics. The cloning of genes involved in the biosynthesis of destruxins would enable the construction of mutant strains defective for a specific biosynthetic enzyme and presumably disrupt toxin biosynthesis. Isogenic toxin-producing and toxin-less mutant strains could then be compared against a variety of insect hosts.

Strain improvement

M. anisopliae and *B. bassiana* have no known sexual stages although genetic recombination through the parasexual cycle has been demonstrated for both (Messias & Azevedo, 1980; Al-Aidroos, 1980; Paccola-Meirelles & Azevedo, 1991). The essential features of the parasexual cycle are the formation of at least a transient heterokaryon by hyphal anastomosis or protoplast fusion, rare fusions between dissimilar nuclei to produce a heterozygous diploid, and haploidization, presumably by a process of mitotic non-disjunction. Genetic recombination can occur by mitotic crossing-over between homologous chromosomes and by the random segregation of chromosomes during haploidization.

Parasexual recombination has been considered as a possible method of genetic manipulation for strain improvement in *M. anisopliae*. Heale *et al.* (1989) reported a parasexual cross between the auxotrophic mutants derived from pathogenic wild type strains, differing in sporulation rate and pathogenicity towards *Nilaparvata lugens* (Stål) (Hemiptera: Delphacidae). Two of the haploid recombinants selected from this cross combined high pathogenicity and sporulation rates. This is in contrast to parasexual crosses between isolates of *Verticillium lecanii* (Zimmermann) Viégas (Drummond & Heale, 1988) and the plant pathogenic species *V. albo-atrum* Reinke & Berthold and *V. dahliae* Klebahn (Clarkson & Heale, 1985; O'Garro & Clarkson, in press) where recombinants were generally of low pathogenicity. A possible explanation is that parasexual recombination between isolates that are normally reproductively isolated, disrupts adapted complexes of pathogenicity genes resulting in weakly pathogenic haploid recombinants.

Genetic engineering offers an exciting alternative approach to strain improvement. The cloning of genes from entomopathogenic fungi whose products are essential for pathogenicity or which contribute quantitatively to virulence may provide the basis for rational strain improvement. Increasing gene copy number and location are consequences of genetic transformation (Bernier *et al.*, 1989) which may result in over-expression of a pathogenicity gene. Gene expression could also be modified by transformation with gene constructs containing the coding region of a pathogenicity gene fused to a strong promoter. Genetic transformation will also provide a tool for moving genes from one entomopathogenic species to another. What is unclear at this stage is how legislation governing the release of genetically manipulated organisms will develop.

Molecular markers for the characterization of entomopathogenic fungi

The genetic characterization of wild-type strains of entomopathogenic fungi can provide information about variation and population structure. It may also be possible to identify individual isolates by a unique set of genetic characteristics, although this could not be done where isolates were actually clones of the same genotype, as may occur particularly in epizootics. Two types of naturally occurring genetic markers which have been used extensively for plant pathogenic fungi are mating type and specific virulence, but these are not generally applicable to entomopathogens. Molecular markers have several advantages, including co-dominance, selective neutrality, and abundance (Michelmore & Hulbert, 1987). Variations in soluble protein patterns and isozyme polymorphisms detected by electrophoresis have proved useful in a range of fungi. De Conti *et al.* (1980) examined the electrophoretic variation in esterases and phosphatases in 11 wild type isolates of *M. anisopliae* from Brazil and were able to distinguish 5 patterns for esterase. In a study of both *M. anisopliae* var. *anisopliae* Tulloch and the large spored *M. anisopliae* var. *majus* (Johnston) Tulloch, Riba *et al.* (1986) demonstrated that var. *majus* isolates were relatively homogeneous for isozyme profiles but that var. *anisopliae* isolates were highly polymorphic. Isozyme polymorphisms have also been studied in *B. bassiana* (Mugnai *et al.*, 1989).

Techniques which directly investigate genetic variation at the DNA level are rapidly gaining acceptance as the most appropriate tools for studying fungal populations. Several of these will be discussed.

Restriction fragment length polymorphisms (RFLPs) of nuclear and mitochondrial DNA

RFLPs may be detected following digestion with an appropriate type II restriction endonuclease and separation of the DNA fragments by agarose electrophoresis. Differences between isolates in DNA sequence may alter the restriction sites for endonucleases resulting in DNA fragments of varying lengths. For fungal mitochondrial genomes which are usually small (19–121 kb) and exist in relatively high copy number (Grossman & Hudspeth, 1985) RFLPs are usually easy to detect. Total DNA of the fungus is extracted and then the mitochondrial DNA separated from nuclear DNA by centrifugation in CsCl gradients containing bis-benzimide (Garber & Yoder, 1985). The small size of mitochondrial genomes results in a simple banding pattern when cut with restriction endonucleases. Mitochondrial RFLP markers have been used in a number of plant

pathogenic fungi although the extent of variation differs considerably.

Although variation may sometimes be detected between strains in ethidium bromide stained gels of restriction endonuclease digests of total genomic DNA (e.g. Coddington *et al.*, 1987), labelled DNA probes are normally required. Total genomic DNA is extracted and digested with a restriction endonuclease. The complex array of DNA fragments are then size fractionated by agarose gel electrophoresis and transferred to a nylon or nitrocellulose filter (Southern blotting). The filter is then probed with a radioactive or non-radioactive labelled DNA probe and visualized by autoradiography or development of the filter, respectively. The number of bands observed depends on the copy number of DNA sequences homologous to the probe and the number of restriction sites within the sequence.

Various types of DNA probe have been used to detect RFLPs, e.g. random genomic clones containing single copy (Hulbert *et al.*, 1988) or repetitive sequences (Kistler *et al.*, 1991), rDNA (Garber *et al.*, 1988) and oligonucleotide probes (Mayer *et al.*, 1991). A. Jessop, I. Paterson & Clarkson (unpublished data) have recently used random genomic clones in the lambda vector EMBL3 to detect RFLPs in *M. anisopliae*.

Polymerase chain reaction (PCR) methods

The polymerase chain reaction (PCR) is an *in vitro* method for amplifying DNA sequences using very small amounts of target DNA (White *et al.*, 1989). The double strand target DNA is denatured by heating briefly at high temperature (ca. 95°C) and the oligonucleotide primers are allowed to anneal to their complementary sequences at low temperature (dependent on probe length and composition). DNA extension from the annealed primers proceeds using dNTPs and a thermostable polymerase, e.g. Taq polymerase, at 72°C. Repeated cycles of denaturation, annealing and extension lead to the rapid amplification of target DNA sequences which can usually be resolved by conventional agarose or polyacrylamide gel electrophoresis.

It has been demonstrated recently that PCR can be used to identify DNA sequence polymorphisms by the amplification of random DNA segments with single oligonucleotide primers, of arbitrary sequence (Williams *et al.*, 1990; Welsh & McClelland, 1990). This randomly amplified polymorphic DNA (RAPD) can be visualized on ethidium bromide stained agarose gels as discrete bands which may provide a unique genetic 'fingerprint' for a particular genotype. This technique is being applied to a wide range of organisms, including *Metarhizium* spp. (R. Milner, pers. comm.).

Electrophoretic karyotyping

Pulsed field gel electrophoresis (PFGE) is capable of separating DNA molecules ranging from about 20 kb to 12,000 kb (Mills & McCluskey, 1990). Schwartz & Cantor (1984) first applied this technique to the separation of the chromosomes of *Saccharomyces cerevisiae* Meyer ex Hansen. In conventional gel electrophoresis DNA molecules migrate in a single electric field. The migration rate of molecules less than approximately 20 kb is proportional to their size because they are sieved. Larger molecules are not sieved effectively and consequently migrate at similar rates. PFGE utilizes alternating electric fields which force the DNA molecules to change direction. The time required for a DNA molecule to change direction is size dependent with larger molecules requiring more time to re-orientate. The duration of each alternating electric field (or pulse) determines the resolution. Total run times may last several days.

The resolution of PFGE has been improved by modification to the original apparatus and protocol. One commonly used method is contour-clamped homogeneous electric field (CHEF) gel electrophoresis where a hexagonal array of electrodes produces a uniform electric field across the gel resulting in straight running lanes and good resolution. DNA is usually prepared from fungi by embedding protoplasts in blocks of low melting point agarose. These are mixed with a detergent (e.g. SDS), EDTA and proteinase K. The agarose blocks containing 0.5–20 μg DNA are then inserted into a well of the agarose gel and sealed with agarose.

PFGE has not been reported for entomopathogenic fungi although it has been used to study the molecular karyotypes of a wide range of plant pathogens and other fungi of commercial importance. Studies with field isolates of plant pathogenic fungi have revealed a surprising degree of variation in both chromosome number and length (e.g. Kinscherf & Leong, 1988; Mills & McCluskey, 1990) suggesting that the electrophoretic karyotypes may prove to be valuable molecular markers for entomopathogenic fungi.

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Selection and characterization of strains of *Metarhizium anisopliae* for control of soil insects in Australia

R.J. Milner

CSIRO, Division of Entomology.

Abstract

The most successful microbial pesticides are based on the bacterium, *Bacillus thuringiensis* (Berliner), with sales of over US$40M. This success has been slow to come and in part has been due to the screening of large numbers of isolates to find the most pathogenic. Recent extensions of the host range have come from searches of habitats of particular pest species e.g. var. *israelensis* from the mud of mosquito pools. In Australia, a semi-selective medium has been used to isolate the insect pathogenic hyphomycete, *Metarhizium anisopliae* (Metschnikoff) Sorokin, from soil and termite mound material. Isolates vary enormously in colony morphology but this character rarely correlates with pathogenicity. Other more sensitive methods such as the analysis of nuclear DNA using polymerase chain reaction (PCR) technology is showing promise for identification of individual isolates (strains). A three tiered screening strategy which has been adopted to select the most promising isolates, firstly for control of sugarcane scarabs and more recently for termites, is described. Two isolates pathogenic for a wide range of sugarcane scarabs and another, more specific, isolate have been successfully field tested and are now being commercialized. For termites, one isolate effective against both of the main Australian pest genera has been selected and is now being field tested.

Introduction

The world's most successful microbial pesticides are based on the bacterium *Bacillus thuringiensis* (B.t.), with annual sales estimated to be over US$40M. What lessons can be learnt from this success? Firstly, success has

been and continues to be, a slow process, with the first products on the market as long ago as 1935. Secondly, and central to this workshop, success has been based on the discovery of effective isolates, in particular, the discovery by Dulmage of the HD1 strain which is highly pathogenic for *Trichoplusia ni* (Hübner) (Lepidoptera: Noctuidae) (Dulmage, 1970). This transformed the product from a curiosity to a significant commercial product, and the HDI strain still forms the basis of products such as Dipel today. Subsequent searches in the habitat of the target pest revealed firstly B.t. var. *israelensis* Goldbert & Margarlit (Goldberg & Margarlit, 1977), then B.t. var. *tenebrionis* Krieg, Huger, Lagenbruch & Schnetter (Kreig *et al.*, 1983), now both successful commercial products, and very recently in New Zealand, isolates effective against the New Zealand grassgrub, *Costelytra zealandica* (White) (Coleoptera: Scarabaeidae) (Chilcott & Wigley, 1990). This search for new isolates with novel activities has become almost obsessive, with well over 25,000 isolates now in culture collections around the world. Finally, bioassay against the target insect is still the only way to determine the activity of these new isolates. I suggest that the task of developing an effective microbial pesticide for locusts and grasshoppers is going to be slow, and that a large number of isolates will have to be bioassayed before success is achieved.

In this short paper I will describe the work undertaken in my laboratory in Canberra to identify, and to characterize, isolates of the hyphomycete fungus *Metarhizium anisopliae* (Metschnikoff) Sorokin, for control of scarab pests of sugarcane (Milner, in press) and termites. In both cases it was necessary to find isolates which were highly pathogenic to the target under field conditions, effective against several related species of target pest, and had temperature profiles which matched the prevailing field conditions. Our approach has been firstly to assemble as large a library of isolates as possible, mostly from the target pest or its immediate environment, then to bioassay them against the pest using a three tiered strategy, and finally to compare the other relevant characteristics, such as temperature profiles, of a small number of effective isolates. It is rarely possible to evaluate more than about three (ideally just one) isolates in large-scale field trials.

Isolate acquisition

There are three main sources of isolates: culture collections such as the American Type Culture Collection or that of the International Mycological Institute (CABI); isolation from the environment of the target insect; and direct isolation from the target insect itself. If one is lucky enough to find a field epizootic and to isolate the fungus directly then this isolate(s) is likely,

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but not certain, to show a high degree of pathogenicity. For soil insects, this medium is the reservoir for all pathogens and not just *M. anisopliae*; thus it seems logical that effective isolates can be obtained in this manner. Effective isolates are rarely to be obtained from culture collections, in my experience.

We have found 'Veens Medium' (Milner & Lutton, 1976) to be extremely effective as a semi-selective isolation medium. It is able to detect levels of conidia down to 10^3/g soil and when compared with other selective media recovers a wider variety of isolates (A. Rath, pers. comm.). We generally isolate from a composite soil sample taken from 2–6 cm below the surface, the depth at which most conidia are found under natural conditions. A 1 g sample of soil is sonicated briefly in a 0.05% Tween 80 solution and 100 µl of the resulting suspension is spread over the surface of a Veens medium plate. The characteristic colonies of *M. anisopliae* start to sporulate after 2 or 3 weeks' incubation at 25°C. These can then be removed and subcultured into SDAYE for testing.

M. anisopliae is surprisingly easy to find in a wide range of habitats. Undisturbed pastures in wet climatic zones where Scarabaeidae are common certainly provide large numbers of isolates (Table 1). Under these conditions it is not unusual to find fields where the average density of conidia exceeds 10^6/g of soil. The fungus is much more scarce in frequently cultivated fields in relatively dry hot climates such as groundnut fields in Queensland and Myanmar. Under these conditions it is rare to find more than 2×10^3/g. Similarly in termite mounds the fungus is not uncommon but is always at a low density.

Table 1. Summary of the results of selective isolation of *Metarhizium anisopliae* from soil in natural habitats in Australia and Myanmar (Burma).

Area	Crop	No. sites	No. positive sites
Mackay, Qld	sugar	17	9
Bundaberg, Qld	sugar	11	8
Cairns, Qld	sugar	21	19
Yeppoon, Qld	pineapple	4	3
Busselton, WA	potatoes	11	10
Pemberton, WA	potatotes	20	9
Ebor, NSW	pasture	9	8
Kingaroy, Qld	peanuts	20	8
Myanmar	peanuts	21	10
Australia	termites	469	65

Isolate variability

The most obvious feature of *M. anisopliae* on isolation is the extreme diversity of colony types, viz. the colour of the conidia, the pattern of conidiation, the degree of sporulation, the colour and the texture of the aerial mycelium, the rate of growth, the level of pigmentation of the medium, and so on. It is possible to erect different morphological types and designate each isolate to a particular type. However, other characteristics (in particular pathogenicity) do not correlate with colony morphology and as the number of isolates to be so designated increases so does the difficulty of placing each isolate into a category.

Spore size also varies, but apart from the well-established (and extreme) difference between *M. anisopliae* var. *majus* (Johnston) Tulloch and *M. anisopliae* var. *anisopliae* Tulloch, this characteristic is not particularly useful. Isozymes have been extensively used to identify isolates and can be extremely useful in separating isolates which are otherwise very similar. However, this technique has two serious drawbacks. Firstly, the banding, especially for esterases which are the most useful marker enzymes, can be unstable with some bands absent for no apparent reason in some runs. Secondly, while differences are important and genuine, similarities are not. Thus, two distinct pathotypes can have identical isozyme patterns.

The most powerful and reliable way to characterize isolates is by means of DNA analysis using modern polymerase chain reaction (PCR) technology (see Clarkson, these proceedings). Recent unpublished work in my laboratory (F. Driver, J. Curran & R. Milner, unpublished data), has revealed significant sequence variation within *M. anisopliae*, and this has been used to develop a probe specific to some isolates of var. *anisopliae*. It is expected that, as our knowledge and understanding of the genome increases, we will be able to produce probes which have a greater degree of specificity.

Screening against scarabs

The first tier is a maximum challenge test which is extremely rapid and uses very few of the pest insects – an important factor because scarabs cannot be reared in the laboratory and are laborious to collect from the field. The larvae were rolled in pure dry conidia and incubated on moist tissue paper in individual chambers at 25°C for about 14 days. Under these extreme conditions 'good' isolates will kill 100% of the grubs and the majority of infected grubs will produce copious quantities of new conidia. Surprisingly,

many isolates from the soil fail to kill under these conditions while some isolates obtained from the target pest do not kill more than 50% of the insects.

Isolates which were most effective in the Tier 1 test were then assessed using a quantitative bioassay. The larvae were dipped into suspensions of different concentrations of conidia and then incubated on moist tissue paper for 2 to 4 weeks at 25°C (Tier 2). Under these conditions the 'good' isolates had an LC_{50} (lethal concentration) below 10^5 conidia/ml.

Finally, a few really promising isolates were compared under simulated field conditions using soil treated with conidia or dosed with formulated fungus. The disease develops more slowly in soil and these tests usually continued for 7 weeks. The most promising isolates from this Tier 3 test were used for field trials.

In our work on sugarcane scarabs, this scheme was not applied rigorously because new isolates, often from field epizootics, became available during the testing and so were included in the then current Tier 3 tests without any prior testing. However a total of 115 isolate/target combinations were tested in Tier 1, 26 in Tier 2 and 40 in Tier 3 (Table 2). As a result two isolates with high pathogenicity for a wide range of sugarcane scarabs (FI147 and FI153) and one with a more restricted hose range (FI114) were selected for field trials. These isolates are now being commercialized under licence by Incitec Ltd.

Screening against termites

Termites are fragile insects, sensitive to handling and wetting. Thus, a dosing technique was devised to reduce these problems and to take advantage of the termites' natural grooming behaviour. For this method, a single worker termite is rolled in a dust of conidia of *M. anisopliae* and then placed in a 70 ml plastic container with 50 healthy workers. The treated carrier termite is groomed by the test termites and the mortality of this test group is determined after 4–10 days at about 27°C. By diluting the fungal conidia with talc this bioassay can be made quantitative. The dose is estimated from haemocytometer counts of conidia washed off the carrier termite.

In these termite assays, the Tier 1 testing consisted of both a high dose (1:10, conidia: talc) and a low dose (1:1000) together with controls. There are large numbers of termites available from the field consequently 150 workers (3 replicates of 50) could be tested per treatment. The most promising isolates progressed to a Tier 2 test which was a quantitative bioassay with 5 decimal doses. Finally, the best 10 isolates were tested against both soldiers and workers under more realistic conditions (25 g colonies in glass jars). In these Tier 3 tests, the termite colonies were dosed by adding 250 carrier termites treated with pure conidia, and the mortality was evaluated after 4 weeks at 26°C.

It was found that all isolates were at least moderately pathogenic and a large number of isolates were promising in Tier 1 tests (Table 3). In general the 'lower' termites, of the genus *Coptotermes*, were more susceptible than the 'higher' termites, *Nasutitermes*. Some 26 isolates were retested in Tier 2 bioassays and these also showed only small differences (Table 4). The final selection of 10 isolates for Tier 3 testing was somewhat arbitrary and the results varied depending on the particular colony of termites used for the testing. However, isolate FI610, derived originally from termite mound material, has generally performed well and has thus been chosen for the field trials which are currently in progress.

Table 2. Summary of Tier testing of isolates of *Metarhizium anisopliae* against seven species of cane grub (Coleoptera: Scarabaeidae).

Species	No. isolates tested			Best isolates
	Tier 1	Tier 2	Tier 3	
1 yr life cycle				
Lepidiota consobrina Girault	1	4	2	FI147;153
L. squamulata Waterhouse	10	0	0	
Antitrogus mussoni (Blackburn)	18	4	21	FI147;153
2 yr life cycle				
A. parvulus Britton	27	4	2	FI114;147
L. frenchi Blackburn	34	8	2	FI147;153
L. negatoria Blackburn	25	3	13	FI147
L. noxia Britton	0	3	0	FI147(?)

Table 3. Summary of results of Tier 1 testing of isolates of *Metarhizium anisopliae* against 3 species of pest termites.

Termite species	No. isolates	High dose mort. > 80%	Low dose mort. > 40%
Nasutitermes exitiosus (Hill)	93	33	16 (7)*
Coptotermes frenchi (Hill)	26	24	7 (7)
C. acinaciformis (Froggatt)	49	48	19 (19)
	24	16	0 (0)

*figures in brackets assessed after 4 days; others assessed after 8 days.

Table 4. Summary of results of Tier 2 testing of isolates of *Metarhizium anisopliae* against 2 species of pest termites.

Species tested	No. isolates	$LD_{50} \times 10^1$ spores/termite		
		1–15	<15–30	<30
Nasutitermes exitiosus (Hill)	26	11	9	6
Coptotermes acinaciformis (Froggatt)	26	26	0	0

Conclusions

The first priority in developing mycoinsecticides is to select an isolate which is highly pathogenic to the target pest or pests. In many situations, such as control of scarab larvae in Australian sugarcane fields, it is certainly desirable, and perhaps essential, that an isolate is found which controls a number of species from related genera. In order to find the best isolate, it is important that as wide a library of isolates as possible is tested and that this testing is by bioassay, ultimately under conditions as close as possible to those in the field. Isolates obtained directly from field epizootics on the target pest(s) have been found to be best, although isolates may vary in their pathogenicity even from the same epizootic.

The three tiered assay strategy has been found to be very useful in quickly assessing the efficacy of a large number of isolates. Three isolates (FI114, FI147 and FI153) with promise for control of sugarcane scarabs have been identified, and these are now being commercialized following successful field trials. Termites were found to be less variable than scarabs in their responses to various isolates. However, one isolate, FI610, which is highly pathogenic for both *Coptotermes* spp. and *Nasutitermes* sp., has been selected for detailed study and field trials. It has been found that DNA analysis using PCR technology is a valuable and sensitive technique for determining genetic differences between isolates. The isolates selected for field trials are all distinct and it is hoped to develop specific DNA probes for use in field trials.

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Exploration for locust and grasshopper pathogens

C. Kooyman[1] & P. Shah[2]

[1]*Département de Formation en Protection des Végétaux, Niger:*
[2]*International Institute of Biological Control.*

Abstract
Current availability of pathogens from Orthoptera, particularly entomopathogenic fungi, and knowledge of their impact on field populations are very limited. The exploration methods for insect pathogens used in the IIBC/IITA/DFPV collaborative research programme for the biological control of locusts and grasshoppers are described. A combination of direct and indirect methods is used, including point surveys, site monitoring and networking. Yield is maximized in several ways, for example by stressing insects in cages at high densities, by exposing insects to infection from soil and by using selective media for isolation from soil. Monitoring of mortality factors over time will reveal the extent of pathogen impact on acridid populations.

Introduction

In the IIBC/IITA/DFPV collaborative research programme for the biological control of locusts and grasshoppers, the biological agents which are considered to have the greatest potential for development for control are entomopathogenic fungi in the genera *Metarhizium* and *Beauveria* (see Greathead; Prior; Lomer *et al.*, these proceedings). Much emphasis has been placed on the need to search for suitable genotypes in the environments where the target pest species occur, despite the apparent unsuitability of such hot and arid conditions for the existence of fungi. The reasons for this are two-fold.

Firstly, it is believed that isolates obtained from these environments

will be better able to tolerate the harsh climatic conditions, especially high temperatures and high levels of damaging radiation, which occur in many of the areas where control must be carried out. Secondly, there are no clear guidelines at present on quarantine protocols for the movement of isolates of these fungi around the very wide geographical range over which desert locust (*Schistocerca gregaria* (Forskål)) control may be required, and it is therefore desirable to have isolates available from as wide a range of potential user countries as possible.

Although the climate in the more arid areas of Africa and Asia where grasshoppers and locusts are a problem is extremely dry for a large part of each year, favourable conditions for fungal growth do occur during the brief rainy season. These areas have been little explored for insect pathogens, and even short surveys may result in new discoveries. For example, during a six week study near Mourdiah, Mali, in 1990, a *Sorosporella* sp. (Deuteromycotina: Hyphomycetes) was found causing significant mortality in *Kraussaria angulifera* (Krauss). This fungus had not been recorded previously at this site despite several years' observations and may be a new species (P. Shah, unpublished data). Thus during a survey it is possible, and indeed likely, that new species will be discovered and it is also possible to study the impact of pathogens on populations.

Search range

The need for exploration is obvious if the first reason (above) is accepted because few isolates of entomopathogenic fungi which meet these requirements are available from existing collections. The isolates available from two major collections, the USDA Agricultural Research Service Collection of Entomopathogenic Fungi (ARSEF) and the International Mycological Institute (IMI) are listed in Table 1. The number of isolates of *Metarhizium* spp. and *Beauveria* spp. in these collections from the major outbreak areas of locusts and sahelian grasshoppers was extremely small when the project began, even if all hosts, including non-orthopterans, were included and the number of available isolates from orthopteran hosts world-wide was also very low. Although much of the sahelian zone is under-recorded in mycological collections, the same cannot be said of such habitats as rice fields where extensive collections have been made and grasshoppers are common. This suggests that infections by entomopathogenic deuteromycetes in orthopterans are either genuinely rare, or difficult to find.

The current surveys are attempting to concentrate on the areas where desert locusts are found during recession, despite the political difficulties involved in carrying out research in many of these areas: the Red Sea coast of Eritrea, Sind Province on the Pakistan–India border, the Tibesti

Table 1. Total numbers of isolates of *Metarhizium* and *Beauveria* spp. held in the collections of the United States Department of Agriculture and the International Mycological Institute (sources: Anon., 1988; Humber & Soper, 1986)

Species	Number of isolates held			
	All locations	Africa	Arabia	Pakistan/India
USDA (ARSEF)* **				
M. anisopliae var. *anisopliae* Tulloch	265	0	0	6*
Orthoptera	16	0	0	0
M. flavoviride W. Gams & J. Roszypal	45	0	0	0
Orthoptera	1	0	0	0
B. bassiana (Balsamo) Vuillemin	410	32**	0	0
Orthoptera	6	0	0	0
IMI* **				
M. anisopliae var. *anisopliae*	30	1****	0	1
Orthoptera	1	1****	0	0
M. flavoviride	0	0	0	0
Orthoptera	0	0	0	0
B. bassiana	14	0	0	0
Orthoptera	0	0	0	0

*From *Nilaparvata lugens* (Stål) (Homoptera: Delphacidae)
**From *Sitona discoideus* Gyllenhall (Coleoptera: Curculionidae)
***Only those isolates listed in Humber & Soper (1986) and Anon. (1988) are considered here: other isolates are held in Reserve (restricted access) at IMI including IMI 330189, collected by DFPV Niger in 1988 from *Ornithacris cavroisi* (Finot) (Orthoptera: Acrididae) and IMI 324673, collected by B. Nyambo in 1987 in Tanzania from *Zonocerus elegans* (Thunberg) (Orthoptera: Acrididae).
****From *Schistocerca gregaria* (Forskål) (Orthoptera: Acrididae), collected by F.L. Balfour-Browne in Ethiopia in 1959

Mountains on the Libya–Chad border and the Tamesna region of the Ahaggar Mountains on the Niger–Algeria border have all been difficult or impossible to visit during recent years. The reasons for seeking pathogens in these areas have been discussed elsewhere (see Prior, these proceedings); it is hoped that genotypes of entomopathogenic fungi from these areas will combine virulence with the necessary ecological adaptations for survival.

Direct survey methods

Point surveys

This is the classical method of stopping at intervals along a road or a transect. Infected orthopterans are often slow and easy to catch. Those killed by fungus can occasionally be found attached to the tips of grass stems or branches: those infected by 'summit disease' (*Entomophaga grylli* (Fresenius) Batko) are commonly found in this position. Areas for survey are chosen on the grounds of previous rainfall and/or reports of high population densities. In this way, many sites can be visited increasing the chance of finding diseased insects. On the other hand, pathogen incidence is transient and there is a danger of not finding any infected cadavers.

Site monitoring

Regular sampling of chosen sites over a longer period allows a study of the development of pathogens and other natural enemies in a host population, thereby combining collection of pathogens with studies on their ecology and impact. The method is intensive and complementary to the extensive nature of point surveys: it can only be effective if prior knowledge of the site, gained by point surveys, indicates a high host population is likely.

Indirect survey methods

Networking

A network has been created within the sahelian countries and some other countries in West Africa. In each country, the national crop protection service has designated a co-ordinator from their staff, who organizes the search for pathogens by field staff and farmers (village brigades). He makes regular field trips and distributes information about collecting, preserving and sending specimens. In the beginning, there was a lack of interest in contributing to this network and this was addressed in 1991 by offering cash incentives. The co-ordinators are paid an allowance plus money to buy fuel for the field trips. People who find grasshoppers infected by a fungus are paid a reward of CFA FN 10,000 or equivalent in appropriate local currency.

Articles

A short article requesting people to send diseased grasshoppers has been sent to a number of journals with a distribution in the survey and network areas. So far, this has been published in SAS (Surveillance des Acridiens du Sahel) Bulletin of PRIFAS and by the newsletter Nouvelles de PRONAT, Dakar, Senegal. A reward is again offered to those people sending in specimens with suitable pathogens.

Maximizing the yield of pathogens

All of the methods outlined above have their shortcomings. Therefore, different methods are combined as far as possible. Obviously, the two indirect methods can be employed together with the direct ones. The extent to which one or the other of the direct methods is employed depends on local circumstances. For example, in known breeding grounds such as the Tokar Delta of Sudan where there is a high probability of finding breeding populations of desert locust, the emphasis would be on site monitoring. On the other hand, in West Africa where grasshopper populations must be examined over a very wide area, point surveys are necessary first, followed by site monitoring only when areas with a high potential for pathogen development are discovered.

Methods for improving pathogen yield include:

1. choosing sites with a history of high and/or diverse populations
2. concentrating on the more humid areas within these sites, and during the wetter parts of the year
3. capturing many grasshoppers at a site, keeping them in cages and checking for the development of symptoms of disease. By keeping densities and humidity artificially high in cages, stress will be increased and the development of incipient infections will occur more rapidly
4. baiting pathogens from the soil, using either well-proven susceptible insects such as greater wax moth larvae *Galleria mellonella* (Linnaeus) (Lepidoptera: Pyralidae) (Zimmermann, 1986) or local grasshoppers. The latter are more likely to yield pathogen genotypes with useful levels of virulence to orthopteran pests
5. direct isolation from soil, using one of the many selective agar media available (Veen & Ferron, 1966; Doberski & Tribe, 1980; Beilharz *et al.*, 1982; Chase *et al.*, 1986; Bååth, 1991; Sneh, 1991).

Conclusions

Very few methodical and extensive surveys for entomopathogenic fungi have been reported in the literature, and those isolates available in the world's collections have been obtained largely by chance. The majority of isolates have come from insects living in humid conditions, where fungal infections may often be common and the outgrowth of the sporulating mycelium on the exterior of the cadavers makes them obvious to collectors. By comparison, in dry environments no external growth occurs, and the fungal origin of infections is concealed unless the cadaver is examined internally. This lack of external symptoms may have led to an underestimation of the importance of fungal infections in dry environments. For example, two isolates of *M. flavoviride* W. Gams & I. Rozyspal held by IIBC are highly virulent to desert locusts and are under investigation in the current programme. Both were obtained from grasshoppers collected in the field (in Niger and Tanzania) where only internal growth and sporulation were observed. In the current survey programme the mortality due to these fungi in field populations in arid environments will be investigated and may be found to be higher than previously expected.

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Panel discussion: precis

Following Dr Prior's talk, Dr Raina asked about the host range of pathogens in general compared with chemical pesticides. Dr Prior replied that there was no real comparison, as chemical pesticides are usually general poisons, whereas pathogens have an inherently much greater specificity.

Dr Milner asked Dr Charnley about the role of destruxins in host death. Dr Charnley replied that Lepidoptera muscle reacts by paralysis which can lead to death, but that this effect appears to be confined to Lepidoptera. In response to a question from Dr Goettel about synergism between insect growth regulators (IGRs) and pathogens, Dr Charnley replied that this was the subject of an ongoing research programme at Bath University, and commented that during moults the gut flora was not lost although the gut lining was replaced. Dr Charnley further commented that the activity of cuticle-degrading enzymes varied with temperature; thus any insect control systems which relied on these enzymes would be temperature-dependent in their effect. Concerning IGRs, Dr Matthews commented that the diflubenzurons were originally developed as fungicides. Was there any effect on fungal control agents? Dr Charnley replied that the second generation chitin synthesis inhibitors had very little effect on fungal spores.

Dr Lomer asked for clarification from Dr Streett of the doses of poxvirus used to kill *Oedaleus senegalensis* and he replied that 10^6 virus particles per insect caused 90% mortality, while 10^3 caused 30–50%, both in 10 days. The viruses were found in early season populations in most places searched. Infection rates varied from below 1% to 8%. Answering a question from Mr Mouhim, Dr Streett gave more details of the conditions under which the entomopox viruses were tested. Mainly US grasshopper species, not *Oedaleus senegalensis*, were used. Insects were treated in the

third instar at 30°C. The virus survived for up to 8 h at 50°C.

Dr Prior called for comments on Mr Kooyman's efforts to set up a network of collaborators to look for grasshopper pathogens. Dr Castleton was very much in favour, and mentioned the SAS (Surveillance des Acridiens au Sahel) network established by PRIFAS. Network identity can be strengthened by tee-shirts, caps, etc. He also suggested the preparation of training materials to enable village brigades to be involved.

Dr Henry asked about the feasibility of infecting grasshoppers in locust breeding grounds during recessions, which could then act as reservoirs of infection. Mr Shah replied that no grasshopper or locust species were sufficiently dense in recession areas to support pathogens. Dr Milner commented that locust outbreak areas in Australia were as dry as Oman, but that rains were often dramatic and could lead to outbreaks of *Entomophaga grylli*. This implies that the spores must persist 5–7 years between rains in desert conditions. Although it is difficult to isolate spores from the soil, they must be present. Dr Goettel stressed the importance of variation in isolates, and that different isolates would be needed in different situations. He called for a free exchange of isolates between researchers. Dr Prior endorsed this, and noted the poor representation of African isolates in the world culture collections. Dr Matthews commented that most locust experts agreed that disease epidemics in swarming locusts were very rare, but that in some areas of grassland in Malawi, where small residual locust populations persist, epidemics of fungal diseases had been observed.

Section IV

Mass Production, Application and Formulation

Mass production, formulation and application of fungi as biocontrol agents

B.A. Auld

Agricultural Research & Veterinary Centre, NSW, Australia.

Abstract

Mass production, formulation and application methods are vital steps in downstream development of biopesticides but surprisingly little work has been done in these areas. While submerged culture fermentation is usually regarded as the most economical and convenient method for mass production of biopesticides by industry, it may not necessarily be so in developing countries. Various methods of liquid and solid state culture for fungi as biopesticides are described. Formulation of fungal spores or mycelium as dry powders for shelf life is well established. However the development of rehydrated propagules in liquid formulations for spraying is in the formative stages of research. The use of oils as spray supplements appears a promising means of overcoming dew or high humidity requirements for many fungi. Because of their small size fungal spores can usually be applied with conventional equipment and the range of application techniques is virtually unlimited.

Introduction

The greatest research effort in biocontrol of insects has been with viruses and bacteria although the entomopathogenic fungi are the largest group of insect pathogens. There appears to have been some reluctance to pursue fungi as biocontrol agents because of environmental limitations to their successful use (National Academy of Sciences, 1969). Such difficulties may be overcome with suitable formulations. The inability to produce many biocontrol fungi en masse has also hindered their development as biocontrol agents.

Fungi have been used in attempts to control weeds since the 1950s. Commercial mycoherbicides were produced in the USA in the early 1980s. Several candidate products are now at various stages of development.

Mass production

Submerged culture fermentation

In the developed world at least, industrial fermentation provides the simplest and most economical way to produce large numbers of fungal spores, as existing equipment can be used without modification. The commercial biocontrol agents for weed control, Collego® and Devine® are produced by this method (see Churchill, 1982; Stowell, 1991) as well as *Verticillium lecanii* (Zimmermann) Viégas (as blastospores) for control of aphids (Aphididae) (Mycotal®) and whitefly (Aleyrodidae) (Vertalec®) (Latge *et al.*, 1986) and some strains of *Beauveria bassiana* (Balsamo) Vuillemin for insect control (Thomas *et al.*, 1987).

Some fungi may sporulate even in liquid culture in flasks. Generally agitation is required to dissolve oxygen; the type of agitation may be important and the air/liquid ratio as well as the surface contact area may need to be high. Conical flasks of 50–2,000 ml may be used; the medium is sterilized with the flask, relying on a porous closure, such as cotton wool, to act as a depth filter. Flasks with side indentations to act as baffles will encourage greater turbulence and aeration. Reciprocating shakers are inferior to orbital shakers which can give high oxygen transfer rates. Reciprocating shakers tend to splash the closure, increasing the danger of contamination. Sophisticated shakers in which temperature and light are controlled may be necessary.

A pilot shaker represents the next stage up from shake flasks; it allows greater oxygen input and control as well as control of temperature, pH, agitation and foaming. Oxygen mass transfer is a major problem for aerobic processes as the solubility of oxygen in water is only about 6 ppm. Oxygen transfer can be increased by agitation which minimizes boundary layer resistance and maximizes the surface area for transfer, thus the speed and diameter of the impellor has a big influence on oxygen transfer. The diameter of the impellor should be about one-third the diameter of the reactor. Baffle plates assist turbulence, breaking up the liquid; they should be about one-tenth the diameter of the reactor. Standard industrial reactors are from 1,000 l to 800,000 l. Smaller pilot reactors cannot mimic exactly what larger reactors will do; for instance there is a marked increase in shear at impellor tips but longer mixing times as volume increases. The minimum size of pilot fermentors generally available is 1 l.

Fermentor (or Bioreactor) operation may be batch culture, semi-batch or continuous culture (e.g. see Trinci & Wiebe, 1990). Batch culture is the simplest procedure with less likelihood of contamination. The output of a product such as fungal spores generally follows a sigmoidal curve with lag, exponential, stationary, and perhaps decline, phases. The growth of product can be affected by substrate limitation and inhibition, product inhibition as well as temperature and pH. If growth is limited by the rate of solubility of an essential nutrient it will be linear; for filamentous organisms, growth in the exponential phase is reduced compared to unicellular organisms. For the latter the exponential growth phase can be described by:

$$\frac{dx}{dt} = \mu x$$

where μ is the specific growth rate (h^{-1})
x is the cell (biomass) concentration (g/l)
t is time
for filamentous organisms it is:

$$\frac{dx}{dt} = \mu x^{2/3}$$

as the cell growth is essentially in two dimensions.

Although maximum total number of product – say fungal spores – may be obtained in the stationary phase, spores harvested during the exponential phase may have a higher percentage viability. If this is the case, semi-batch (or fed batch) culture may be worthwhile.

Possibly one of the greatest problems encountered with fermentation will be contamination. Sterilization is a vital part of the process but the degree of sterility required is a function of the end use.

Media for growth of the biocontrol organism should be as simple as possible, utilizing a standard set of inorganic salts and sources of carbon and nitrogen. Production of spores in the fermentor may be enhanced by changes in media components or simply by diluting the medium (Auld *et al.*, 1988). Production may also be increased by beginning the fermentation process with a larger volume or concentration of starter culture than normal. (See Latge & Moletta (1988) for an extended treatment of the production of entomopathogenic fungi in submerged culture).

It may be necessary to screen isolates for spore productivity under fermentation conditions. The most virulent isolates may not be the most productive *in vitro*. Quantities of original single spore isolates should be stored for continued use in larger scale production because of possible loss of virulence through sub-culturing.

Recovery of spores from bioreactors may be a problem with filamentous fungi, requiring large centrifuges to spin off spores. Filtration methods often leave a large number of spores behind in the mycelial mass.

Following recovery of spores from a production process it is usually necessary to dry them for long term storage. It is not always possible to do this and retain viability of the organism. It is for this reason that the mycoherbicide Devine® is sold in liquid form like fresh milk. Drying should be done as quickly as possible under 'clean' conditions to prevent bacterial contamination. Spores may be washed during the collection process but this may reduce infectivity by removing enzymes in the matrix which surrounds many spores (McRae & Stevens, 1990). Inert materials such as kaolin, diatomaceous earth, or silica gel, may be suitable to hasten drying and to act as carriers. Contamination of the spore slurry during this stage may be a problem, so it is preferable to dry spores as quickly as possible.

Some fungi which do not sporulate in submerged culture may produce mycelium which can be dried (Pereira & Roberts, 1990) and applied as fragments or pellets in the field; among them is *Metarhizium anisopliae* (Metschnikoff) Sorokin produced by Bayer, Munich, Germany, as BIO 1020®. Such fungi may sporulate following dew (Roberts & Wraight, 1986; Rombach *et al.*, 1986; Roberts *et al.*, 1987). Walker & Connick (1983) describe the production of sodium alginate pellets of dried mycelium for a mycoherbicide.

Solid substrate fermentation

Solid substrate fermentation is widely used to produce fermented foods in Japan and elsewhere in eastern Asia. Media used contain some liquid; the 'solid' substrate itself may be relatively inert (e.g. paper, wood, vermiculite) allowing for use of defined nutrient levels. On the other hand, some nutritive solid substrates may be available locally at low costs (e.g. coffee pulp, sorghum, straw, groundnut shells). Particle size, moisture content and temperature may all need to be controlled for successful production. Equipment used may be bags, trays or rotating drums.

Industrial submerged culture fermentation production requires considerable capital investment. Production on solid media may be relatively costly in terms of labour and materials in the western industrial environment but not necessarily where labour is less costly and suitable raw material is freely available.

Beauveria bassiana has been produced on solid substrates such as heat sterilized grains in the USSR and the People's Republic of China. In the latter system 500 ml flasks of substrate are used to 'seed' 5 kg lots of steamed grain which is mixed with ten times the amount of wheat bran for fermentation in flat trays or in outdoor pits (Bartlett & Jaronski, 1988). The fungus is produced in liquid surface culture in large inflated plastic bags in Czechoslovakia (Kybal & Vilcek, 1976; Samsinakova *et al.*, 1981). Goettel (1984) has also described a technique for producing fungi using cellophane sheets on bran in autoclave bags. Abbott Laboratories in the USA have made commercial scale up tests for the fungus, using solid substrate to produce either a wettable powder or granular formulation (Bartlett & Jaronski, 1988). R.J. Milner (pers. comm. 1991) has developed a solid substrate fermentation system for *M. anisopliae* using rice, inoculating with conidia and harvesting conidia by a washing technique (Australian Patent PK3451/90).

Two phase systems

A two phase system has been used for *B. bassiana* and *M. anisopliae* production in the USSR, where mycelium produced in deep tank fermentation is allowed to sporulate in shallow open trays (Goral & Lappa, 1973; Roberts & Yendol, 1981). Walker & Riley (1982) described a similar preparation method for *Alternaria cassiae* Jurair & Khan for control of the weed *Cassia obtusifolia*. In Brazil *M. anisopliae* is produced on autoclaved rice or wheat bran in autoclavable plastic bags following inoculation with blastospores produced in liquid shake culture (Aquino *et al.*, 1975, 1977).

Bartlett & Jaronski (1988) highlight the problem of production volume. They cite typical application rates for *Beauveria* conidia per hectare as being about 1×10^{14} (however, Johnson *et al.* (these proceedings) suggest 1×10^{13} conidia/ha for *Beauveria* against grasshoppers); yields for *Beauveria* conidia obtainable from liquid surface culture are 1×10^{14} m^2, submerged liquid fermentation 3×10^{11}/l and 7×10^{12}/kg for solid substrate fermentation. Thus, if submerged liquid production were used a fermentation capacity of over 300 l would be required for each hectare treated. Apart from research on maximizing production per unit volume or area, improved formulation and application techniques may reduce the density of spores required in the field.

Formulation

Formulation has two main aims: to provide an economical and easily useable form of the active ingredient with long shelf life, and if possible to enhance the effectiveness of active ingredient. In many circumstances an aim will also be to minimize the quantity of water required. (Devisetty (1988) has recently reviewed formulations for *Bacillus thuringiensis* Berliner).

An active ingredient may be applied in the dry state as dust or granules or as a liquid or in the presence of liquid. This may be as a wettable powder, in oil, as aqueous concentrate, or emulsifiable concentrate. These

concentrates are diluted by the addition of water. Other techniques such as microencapsulation will not be dealt with here, although they may have scope in the application of biocontrol agents.

Given that a biocontrol fungus can be produced en masse and dried, it can be applied as dry material or formulated as a wettable powder. In both cases clays such as kaolin, silica gel or diatomaceous earths can be used as fillers or carriers. Dusts are prepared so that particle sizes are between 3 and 50 μm in diameter; dusts are, however, vulnerable to wind. Granular formulations are less so, being mostly within the range of 0.3 to 1.0 mm diameter. Granules have been used for mycoherbicide applications by Walker & Connick (1983). Wettable powders may contain dispersing or suspending agents as well as inert fillers and wetting agents. Sodium alginate at 0.2–0.5% of final volume, for instance, will help keep some spore/clay powders in suspension. Wettable powders have been the most common form of microbial formulation (most *Bacillus thuringiensis* is sold in this form); they have advantages of each of storage and transport as well as minimal interaction between spores and other components. Moreover, given that most fungi used for plant and insect control require free water or very high humidity for infection, the provision of water at application is a logical tactic.

Spray supplements

The most important group of spray supplements are surface active agents (surfactants), which include wetting agents, emulsifiers and dispersing agents. They are characterized by having hydrophobic and hydrophilic moieties in their molecular structure. The hydrophobic moieties are generally lipophilic. The relative effect of these two parts of the molecule are described by its hydrophilic – lipophilic balance (HLB). Some examples of extreme HLB values are oleic acid (1) (lipophilic) and sodium lauryl sulphate (40) (hydrophilic). The following gives an indication of HLB values and their application: water in oil emulsifier (3–6), wetting agents (7–9), oil in water emulsifiers (8–18), detergent (13–15) (Becher, 1973). There are thousands of surface active agents produced by hundreds of manufacturers. The choice of surface active agents is apparently limitless but governed by appropriate HLB values.

In addition, sticking agents may be used to improve the adhesion of the spray. Materials used include oils, gelatine and gums. Humectants may also be used to decrease the rate of evaporation. Any solute in water will decrease its rate of evaporation, but propylene glycol and polyethylene glycol have been used specifically for this purpose. It should be noted that a supplement may act in more than one way, say as a humectant, as well as a dispersing agent.

Because many plant pathogenic fungi have a requirement for free water (or dew period) for infection, recent efforts by many mycoherbicide researchers have been directed towards overcoming this dew requirement via formulation; in particular, formulating fungal spores within the aqueous phase of an invert emulsion in oil (or oil mixtures) (Quimby *et al.*, 1989). Although the technique has been shown to overcome the need for dew in some fungi there are serious disadvantages with the method: the amount of oil required adds greatly to the cost of the product, non target contamination by oil, and the difficulty of application of the viscous material.

The lower limit for spore germination for Deuteromycetes infecting terrestrial insects is probably about 92% R.H. (Payne, 1988). The microenvironment of the surface of the insect may provide sufficiently favourable conditions in an otherwise unfavourable environment. The use of non-aqueous carriers such as oil based suspensions has been investigated by some workers (Agudelo & Falcon, 1983) and may show promise for low volume applications of *B. bassiana* (Prior *et al.*, 1988). Prior *et al.* cite work which demonstrated that *B. bassiana* does not actually require high humidity for infection but rather for the production/dispersal of conidia. Glare & Milner (in press) cite evidence for a similar situation with *M. anisopliae*.

The use of oils of vegetable origin at lower concentrations with appropriate emulsifying agents may be a profitable line of investigation. Various oils have been found to decrease inoculum thresholds for fungal infection (Rowell & Olien, 1957) and enhance mycoherbicide performance (Boyette *et al.*, 1991). The resulting mixture of oil, spores, inert carrier and water would be a suspension emulsion.

The use of microemulsions, which are thermodynamically stable transparent emulsions of very fine droplet size (< 0.1 μm), may also have a place in biocontrol formulations. Several surfactants are used to achieve low interfacial tensions. Some microemulsion formulations have already been shown to increase pesticide efficacy (Skelton *et al.*, 1988).

The sensitivity of spores to any ingredient will override other considerations and viability tests must be made continually as a formulation is developed. For instance, Soper & Ward (1981) report variation in the tolerance of *M. anisopliae* to various kaolins and Daoust *et al.* (1982) record the effect of a variety of formulations on the virulence of *M. anisopliae* against mosquito (Diptera: Culicidae) larvae.

Application

Because of their small size, fungal spores can usually be applied in suspension with conventional equipment. Matthews (1983, 1985) has discussed

some particular problems and requirements for spray application in the tropics and developing regions. Obviously equipment will need to be free of residues of fungicides and any other harmful chemicals. Notwithstanding this there may be scope for the addition of low concentrations of conventional insecticides or herbicides to increase the efficacy of a fungus.

The range of techniques available for application therefore is as broad as those available for conventional pesticides and herbicides. These include high volume (about 1000 l/ha), medium (350 l/ha), low to very low volumes (3–150 l/ha), ultra low volumes (0.5–3 l/ha), controlled droplet application and electrostatic spraying. With medium and lower volumes, high powered fans are usually required; with ultra low volumes centrifugal energy may be employed. Symmons *et al.* (1989) describe a vehicular mounted spinning disc sprayer for ultra low volume applications of bendiocarb to desert locust (*Schistocerca gregaria* (Forskål)) nymphs.

Mycoinsecticides, unlike mycoherbicides, can be applied as baits. Bran baits have been a successful method for application of the protozoan *Nosema locustae* Canning for control of rangeland grasshopper (Miller, 1984). Novel methods for application of *B. bassiana* in China have been reported by Soper (1982). These included the use of mortar shells, firecrackers and landmines.

Conclusion

While there are aspects of both mass production and application of fungal pathogens for biocontrol which require a great deal of further work, it is evident that formulation is the area requiring greatest attention. The need for very high humidity or free water for many fungi to infect currently militates against their widespread use. However this requirement should be carefully checked experimentally; it does not appear necessary in *B. bassiana* and *M. anisopliae*. In any event, improved formulations might also mean that lower production per unit volume were required, and lower application concentrations could be used.

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Mass production, formulation and field application of entomopathogenic fungi

M.S. Goettel[1] & D.W. Roberts[2]

[1]*Agriculture Canada Research Station, Lethbridge, Alberta:*
[2]*Insect Pathology Resource Centre, Boyce Thompson Institute, USA.*

Abstract
Successful use of entomopathogenic fungi as microbial control agents of insects will ultimately depend on the use of the right propagule, formulated in an optimum fashion and applied in the field at the right time. For this to be accomplished, detailed knowledge of the host-pathogen interactions and epizootiology is critical. Research and development must then concentrate on developing a method of mass production, formulation, and application, that retains the ability of the pathogen to kill its host. In this brief review, the presently available methods for mass production, formulation, and field application of entomopathogenic fungi are presented. Methods for mass production are *in vivo*, submerged culture, and surface culture. A major current limitation is the lack of readily available formulation technology. Consequently, formulation is frequently simple or nonexistent. Since fungal propagules are microscopic, most can be applied as conventional chemical insecticides; however, since we are dealing with a system involving two living organisms, application methods have to take into consideration the multitude of factors that may affect host-pathogen relationships.

Introduction

Successful development of a pathogen as a microbial insecticide depends on many factors. Not only must the pathogen be virulent against its intended host, but it must also be produced in large quantities, formulated in a way so as to optimize its storage, application, and virulence characteristics, and it must be applied in the field in an economical and efficacious

way. For *Bacillus thuringiensis* Berliner, the attributes of the pathogen have made development relatively easy; fungi on the other hand, are proving more difficult.

Recent reviews on development of fungi for pest control include those of Hall & Papierok (1982), Lisansky & Hall (1982), Latge & Moletta (1988), McCoy *et al.* (1988) and McCoy (1990); reviews on mass production include Soper & Ward (1981) and Bartlett & Jaronski (1988); and reviews on formulation include Soper & Ward (1981), Sawicka & Couch (1983), Most & Quinlan (1986) and Baker & Henis (1990).

In the present paper, we review the production, formulation, and application of entomopathogenic fungi with particular emphasis on how these relate to grasshopper control.

Mass production

Although some potentially useful entomopathogenic fungi are difficult to mass produce, the great majority grow profusely on very simple, inexpensive media. There are several methods available for the mass production of entomopathogenic fungi; *in vivo*, surface, and submerged culture. The method used depends largely on the growth requirements of the fungus in question and the end product desired.

Production *in vivo*

Many obligate entomopathogenic fungi have complex nutritional requirements and are very difficult, if not impossible, to grow in artificial culture. Therefore, the only alternative is to propagate these fungi *in vivo* (Ignoffo & Hink, 1971). However, *in vivo* production is relatively expensive and it is doubtful this method could ever be used economically for mass production of a fungus for use as a microbial insecticide. It must not be disregarded altogether, as commercial production of several grasshopper microbial insecticides is based on the *in vivo* production of *Nosema locustae* Canning.

In vivo production could be used for dissemination of a fungus in a programme of classical or inoculative control; *in vivo* produced inoculum could be introduced into an area where it does not occur or to induce an epizootic at a time when it would not normally occur. For instance, there is still potential for classical biological control through introduction of fungal strains or species from other geographical areas.

Entomophaga grylli (Fresenius) Batko, a fungus responsible for numerous epizootics in grasshoppers worldwide (Skaife, 1925; Pickford & Riegert, 1964; Roffey, 1968; Milner, 1978; Erlandson *et al.*, 1988), is an

example of a fungus which, although it can be grown *in vitro* as protoplasts (MacLeod *et al.*, 1980), produces infective stages only *in vivo*. Successful use of this fungus as a microbial insecticide would require mass production *in vivo*. However, there is still potential in using *E. grylli* for classical biological control; recent work by Carruthers *et al.* (1991) is aimed at exploiting in the USA a particularly virulent pathotype of *E. grylli* imported from Australia.

Submerged culture

Most technology for mass culture of fungi has been developed towards harvesting fungal metabolites using submerged liquid fermentation; however, few entomopathogenic fungi produce infectious propagules in submerged culture. Some exceptions are *Culicinomyces clavisporus* Couch, Romney & Rao (Roberts & Sweeney, 1982), and selected strains of *Aspergillus ochraceus* Wilhelm (Vezina *et al.*, 1965), *Beauveria bassiana* (Balsamo) Vuillemin (Thomas *et al.*, 1987; Rombach *et al.*, 1988; Hegedus *et al.*, 1990) and *Hirsutella thompsonii* Fisher (Van Winkelhoff & McCoy, 1984) which produce conidia in submerged culture; however, these conidia are usually less virulent and are shorter-lived than surface-produced ones (Roberts & Sweeney, 1982). Much more research is needed before this method can be adopted for mass production of conidia of many entomopathogenic fungi.

Most hyphomycetous fungi produce blastospores in submerged culture. Blastospores are short-lived and, consequently, have generally not been adopted for field application (Ferron, 1981; Roberts & Humber, 1981). An exception is *Verticillium lecanii* (Zimmermann) Viégas which has been used successfully as a dried blastospore preparation for control of greenhouse whiteflies and aphids (Lisansky & Hall, 1982). In this case, much of the mortality actually occurs from infection by conidia which develop after the formulation has been applied to the plant surface.

A recent breakthrough has been the development of a method for producing dry, viable mycelium which can be stored under refrigeration (McCabe & Soper, 1985; Andersch *et al.*, 1990). When introduced in the field, particles of dry mycelium rapidly produce infectious conidia following rehydration from dew, rain, or soil moisture. This method can be used with many species of fungi including *Metarhizium anisopliae* (Metschnikoff) Sorokin and *B. bassiana* (Pereira & Roberts, 1990) and has been successfully field tested (Rombach *et al.* 1986a, 1986b, 1988), and a commercial product, Bayer 1020, developed.

Submerged fermentation is most often used in a diphasic method of production; blastospores and mycelia are produced in submerged culture and then transferred to surface culture for production of conidia.

Surface culture

Many fungi grow profusely on damp solid media and produce conidia aerially. Surface culture on solid or semi-solid media is usually best suited for these fungi which include many species in the class Hyphomycetes. *M. anisopliae* is produced commercially in Brazil for control of the sugar spittle bug (*Mahanarva posticata* (Stål)). The fungus is grown on sterilized rice in autoclavable polyethylene bags for 15 to 20 days, dehydrated at 25°C and 35% RH for 72 h, and then milled to obtain a conidial powder (Aquino *et al.*, 1975, 1977; Mendonça, these proceedings). Surface culture methods were developed by Goettel (1984) to produce conidia of several Hyphomycetes, including *B. bassiana*, on bran and by Daoust & Roberts (1983) to produce conidia of *M. anisopliae* on rice.

Mass production of *B. bassiana* using surface culture has been in progress for many years in the USSR and the People's Republic of China. For example, 22 t of Boverin (*B. bassiana* plus inert ingredients) has been produced in a year from a pilot plant in the USSR (Ferron, 1981). In the People's Republic of China conidia are produced on wheat bran, rice powder, compost, or ground corn stalks in flat trays, glass crocks or shallow outdoor pits (Hussey & Tinsley, 1981).

A unique method of surface culture using liquid media has been developed for *B. bassiana* in Czechoslovakia (Kybal & Vlcek, 1976; Samsinakova *et al.*, 1981). Sporulation takes place on the liquid surface in large, inflated plastic tubes. The conidia are harvested by draining the liquid once conidiation is completed.

In the western world, there has been relatively little commercial interest in mass production of *B. bassiana* despite many research programmes involved in the development of this fungus as a microbial control agent. Abbott Laboratories was developing methods for commercial scale-up of *B. bassiana* in the USA, but this programme has recently been abandoned (S.T. Jaronski, pers. comm.).

A recent breakthrough has been the development of a solid culture process for *B. bassiana* by Mycotech Corporation, Butte, MT, (Bradley *et al.*, in press). The process uses a packed bed, solid culture system. A liquid phase is absorbed in a starch-based culture substrate. The gaseous phase remains in the interparticle spaces and the fungus growing on the substrate particles is aerated directly from the gas phase. Using this technique, Mycotech has produced *B. bassiana* at small pilot scale with yields of 10^{13} conidia with fermentor volumes of less than 1 l. Dry conidia powders contain up to 10^{11} spores/g. This process has also been used for production of *Paecilomyces farinosus*, *P. canadensis*, *Nomuraea rileyi* (Farlow) Samson, and *H. thompsonii* and could probably be used for production of most species of hyphomycetous entomopathogenic fungi.

Formulation

A major current limitation in the development of fungi for insect control is the lack of readily available formulation technology. Formulation is frequently simple or nonexistent. Yet, it is through formulation that improved shelf life, persistence, efficacy, and field targeting could be achieved. However, formulating entomopathogenic fungi is in its infancy and poses a very complex challenge. The little technology that does exist is usually proprietary information.

Formulations of fungi may incorporate additives such as wetters, stickers, humectants, UV protectants, and thixotropic agents. Types of formulation include dust, granular, wettable powder, liquid, and flowable formulations. The choice of formulation must ultimately be chosen on the basis of mode of infection, target host habitat, and application method.

B. bassiana has been successfully formulated for grasshopper control in wheat bran baits and oil. However, field results are inconsistent and further studies of formulation effects on host pathogen relationships are needed (see Johnson *et al.*, these proceedings).

Application

Since fungal propagules are microscopic, most can be applied as conventional chemical insecticides. It is somehow perceived that, if enough inoculum is applied, ultimately the hosts will succumb. However, this may be reason why we have not progressed sufficiently in successful field application of entomopathogenic fungi; too much emphasis has been placed on formulating products which can be applied using methodology developed for chemical insecticides and not enough attention has been placed on host-pathogen relationships. Since we are dealing with a system involving two living organisms (i.e. the host and its pathogen), application methods have to take into consideration the multitude of factors that may affect host-pathogen relationships, sunlight, temperature, humidity, host age, inoculum targeting, etc. The method of application is, of course, dependent on the type of formulation available. Since very few formulations are available, most preliminary field testing is done with an unformulated, or crudely formulated active ingredient. However, it is difficult to justify allocating many resources to formulation development unless the active ingredient has been shown to be effective in the field.

Field application must target the inoculum to the host. Since, for the most part, entomopathogenic fungi infect via the external cuticle, the propagule must come into contact with the insect cuticle. Consequently, even subtle differences in host behaviour may greatly affect the efficacy of

an application method. For instance, two species of aphid, *Myzus persicae* (Sulzer) and *Aphis gossypii* Glover, are equally susceptible to *V. lecanii*; however, control of *A. gossypii* was markedly less than that of *M. persicae* when *V. lecanii* was applied according to the manufacturer's recommendations (Hall & Papierok, 1982). This effect was attributed to the higher mobility of *M. persicae* resulting in contact with the fungal spores. This lack of adequate control of *A. gossypii* was overcome by the application of the fungus using a low-volume electrostatic rotary atomizer which optimized deposition of spores on the aphids (Sopp *et al.*, 1989).

B. bassiana produced using Mycotech's surface culture method has been formulated as bran bait or oil formulation (Johnson *et al.*, these proceedings). Oil-formulated conidia can be dispersed in 30–300 μm droplet sizes using hand-held low-volume spinning disc sprayers. Microscopic observation confirmed even distribution of viable conidia in oil droplets. Application rate of 10^{13} spores/ha can be applied in a total volume of 3 l. Although grasshoppers succumb to both formulations in laboratory assays, field trials have given inconsistent results (Johnson *et al.*, these proceedings).

Conclusions

Successful use of entomopathogenic fungi will ultimately depend on the use of the right propagule, formulated in an optimum fashion, and applied in the field at the right time. For this to be accomplished, detailed knowledge of the host-pathogen interactions and epizootiology is critical. Research and development must then concentrate on developing a method of mass production, formulation, and application that retains the ability of the pathogen to kill its host.

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Mass production, application and formulation of *Metarhizium anisopliae* for control of sugarcane froghopper, *Mahanarva posticata*, in Brazil

A.F. Mendonça

Alagoas State Federal University, Brazil.

Abstract

The fungus *Metarhizium anisopliae* (Metschnikoff) Sorokin is used for the control of sugarcane froghopper, *Mahanarva posticata* (Stål) (Homoptera; Cercopidae), on a large scale in Brazil. The present article discusses the background to early work on the fungus, current mass production, substrates and techniques, quality control, formulation, and application.

Introduction

Epizootics of the fungus *Metarhizium anisopliae* (Metschnikoff) Sorokin on the sugarcane froghopper, *Mahanarva posticata* (Stål) (Homoptera: Cercopidae) were first reported in the region of Campos, Rio de Janeiro, in 1964 by Guagliumi (1973).

In the 1960s, with the spread of *M. posticata*, the Commission for the Control of the Cane froghopper in the states of Alagoas and Pernambuco (CCCEAP) was set up to deal with this pest, principally using insecticides. In 1969, during a search for biological control agents, a natural epizootic of *Metarhizium anisopliae* in the root froghopper, *Mahanarva fimbriolata* (Stål) was observed by the author and E.J. Marques. With the collaboration of Maria de Lourdes Aquino of the Agronomical Research Institute (IPA), the first isolation, laboratory culture and experimental applications were carried out.

In 1970, mass production and large-scale application were begun in Alagoas and Pernambuco. Several other Brazilian research organizations

became involved in research on the utilization of *Metarhizium anisopliae* including IAA-PLANALSUCAR and EMBRAPA/CENARGEM.

Mass production – substrates

Liquid media

The use of liquid media for mass production of entomopathogenic fungi is still at an experimental stage. A medium based on molasses, soya flour and mineral salts gave low spore yields (Alves, 1983). Cruz *et al.* (1983) demonstrated satisfactory sporulation in media containing bean broth. In Cuba, 10^{11} spores/l of *Beauveria bassiana* (Balsamo) Vuillemin have been produced in floating cultivation on a medium containing the following ingredients;

Molasses	20–50 g/l
Ammonium phosphate	3 g/l
Copper sulphate ($7H_2O$)	0.5 g/l

Solid media

Rice has been the most commonly used substrate for the mass production of *M. anisopliae* and *B. bassiana* in Brazil. Yields of fungus vary from 10 to 14% of the substrate weight, depending on the quality of the rice. Corn (raw and triturated), potato, beans, sorghums and soya were also tested; all gave lower yields than rice.

Mass production techniques

Various mass production methods have been investigated in Brazil; in this paper, we will only consider those methods currently in use, or likely to be used in the future.

Production in wide-mouthed glass bottles

This method has been widely used in the north-east of Brazil since 1969 for the mass production of *M. anisopliae*, and, on a smaller scale, *B. bassiana*. Currently, this method is not so widely used due to the high cost and difficulty of replacing the bottles.

The following stages are followed:

1. isolation, purification and characterization of the fungus to be produced
2. preparation of the fungus strain inoculum
3. preparation of the inoculum suspensions in 800 ml Roux flasks
4. preparation of the rice medium
5. inoculation of the 500 ml flasks for mass production
6. germination and growth
7. sifting and storage of spores.

This method usually gives a final yield of 10% conidia, but it can be as high as 16%. Contamination does not normally exceed 5% of the bottles. The residual rice is not used again in the laboratory; it is often used in the field in granulated form as an additional source of inoculum (Marques *et al.*, 1981; Mendonca & Rocha, 1987).

Production in plastic bags

This method, using polypropylene plastic bags, is currently the most widely used means of producing *M. anisopliae*. It is also used for the production of *B. bassiana*; in both cases, rice is used as the substrate.

Stages 1 to 4 are identical to those mentioned above:

5. preparation and inoculation of plastic bags
6. germination and growth
7. storage of the bags with fungus.

This method yields 10% of fungus, up to 10^{10} spores/g (Aquino *et al.*, 1977; Mendonca & Rocha, 1987).

With the intention of reducing costs, Biotech (Controle Biologico Ltda) in the state of Alagoas, introduced some innovations in the plastic bag technique, as follows:

1. use of pre-soaked rice instead of dry rice. Yield is increased by enlarging the rice grains and thus increasing their surface area, resulting in economies of up to 50% in the amount of rice used
2. reduction in the size of bags from 40 × 25 × 0.1 cm to 35 × 22 × 0.1 cm with an economy of 20%
3. use of 300 or 400 g of rice per bag instead of 200 g, providing a gain of 50% in the number of bags used.

With these changes, and using the isolate PL 43 of *M. anisopliae*, an average yield of at least 10^{10} spores/g of rice is obtained.

Production in trays (Biomax method)

Developed by the Labormax Produtos Quimicos Ind. e Com. Ltda., in São Paulo, this method is no longer used due to the serious problem of contam-

ination. In Cuba, a similar system is used for the production of *B. bassiana* on barley substrate.

Stages 1 to 4 are the same as those mentioned above (Alves, 1983).

5. preparation and inoculation of the trays
6. germination
7. sifting in vibratory sieve
8. quality control and formulation.

This system shows an average yield of around 8.5%.

Quality control

The mass production of a pathogen requires rigorous quality control. The following factors need to be taken into account when selecting a strain for production; pathogenicity (LD_{50}), conidia production, ultra-violet resistance, conidia viability, etc.

During production, the following quality control tests are carried out at IAA-PLANALSUCAR.

Conidial concentration

Weigh 0.1 or 1 g of the end product; prepare an aqueous suspension containing a dispersant. The number of conidia/ml is determined using a Neubauer chamber. The number of conidia/ml times the volume of dilution will give the number of conidia/g.

Conidial viability

PDA petri dishes plus antibiotic are prepared, conidial suspension is added and the plates incubated for 18–20 hours. 500 spores are checked in two dishes. This test is carried out 0, 30, 45, 60, 75 and 90 days after the fungus is produced. The result is given as a germination percentage.

Purity

Ten microlitres of conidial suspension at a known dilution (500 to 1,000 conidia/10 ml is pipetted on to five dishes of PDA with antibiotic and five without. After four to five days incubation at 27 to 28°C, the colonies of *M. anisopliae* are counted as well as those of contaminating bacteria. The result is given as a percentage of purity.

Virulence (relative dose response)

The LT_{50} of the test strain and the standard is determined by bioassay in the test insect. The relative response (RDR) is determined as follows:

$$RDR = \frac{LT_{50} \text{ of standard}}{LT_{50} \text{ of test fungus.}}$$

Formulation and application

The application of *M. anisopliae* for the control of *Mahanarva posticata* froghopper has generally been carried out using an aqueous suspension obtained by washing the rice and fungus with water. The application rate is 200 g of conidia/ha, corresponding to approximately 10^{12} conidia/ha.

Around 1975, a product called Metaquino was developed in the state of Pernambuco, in which the rice and conidia were ground to a powder. This product was suspended in water at the time of application (Aquino *et al.*, 1975).

Biotech recommends an application rate of 10^{13} conidia/ha. This corresponds to the average production from 10 plastic bags, and this higher dose gives better control in a shorter time (Mendonca & Rocha, 1988).

The volume of suspension applied/ha varies according to the application equipment used:

Ground application	
— back-pack sprayer	400 l/ha
— animal-drawn sprayer	200 l/ha
— motorized sprayer	50 l/ha
Aerial application	20 l/ha

During periods of high fungus production, the residual rice following conidial separation is commonly used as an additional source of inoculum, applied by plane.

The production programme installed in the north-east of Brazil has produced over 50 t of *Metarhizium anisopliae* since 1977. More than 600,000 ha of sugarcane has been treated with this material, making this the biggest microbial control project in the world using *M. anisopliae*.

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The principles of ultra-low volume spraying in relation to the application of microbial insecticides for locust control

G.A. Matthews

International Pesticide Application Research Centre, Imperial College of Science, Technology and Medicine, UK.

Abstract

Droplet size needs to be optimized at 70–100 μm VMD to drift spray locusts using aerial or ground equipment to apply ultra-low volumes (1 l/ha). The concentration of conidia is determined by the dose per insect and number of droplets expected to be deposited on the locusts. Rotary atomizers are appropriate to achieve the narrow droplet spectrum required.

Introduction

Aerial and ground ultra-low volume spraying began with research on application technology for locust control, and especially with the concept of vegetation baiting using a persistent insecticide, notably dieldrin (Courshee, 1959). Using an exhaust gas nozzle to provide a plume of oil-based spray which is deposited over a swath downwind, it was estimated that as a fifth instar hopper could eat approximately 10 cm^2 foliage per day, so a lethal dose of insecticide would be accumulated if there were an insecticide deposit of 4 $\mu g/cm^2$.

Successive track spacings of 200 m have been recommended with the exhaust nozzle drift spraying technique, with droplets having a volume median diameter of the order of 100 μm. The actual distribution of droplets downwind depends on their size and movement relative to the air turbulence, but the aim is to have droplets falling at an angle of 5–7° to the horizontal so that a high proportion of the insecticide is deposited on vertical surfaces, such as the clumps of grass on which the locusts are

feeding, rather than wasting chemical on the exposed soil surface. A peak deposit occurs at a particular distance downwind from a single swath, but with a persistent stomach poison accumulated by the locust hopper, the variability in deposit is relatively unimportant. However, if a contact insecticide is applied the track spacing needs to be adjusted to provide overlapping swaths that overall produce a more uniform deposit.

Johnstone (1991) has developed a computer model to predict the dose received by settled locusts, for example, it predicts that a 100 m track spacing is justified for a spray emitted at 15 l/min flow rate containing droplets with a volume median diameter of 120 μm or less released at a height of 10 m in a wind speed not exceeding 2 m/s.

This paper discusses the relevance of the earlier studies on ultra-low volume spraying to the requirements for efficient transfer of a fungal pathogen to settled locusts.

Droplet size

Theoretical studies on the impaction of droplets on flying locusts suggested that the optimum droplet size was 50 μm (Townsend, 1948), and other studies have confirmed the importance of aerosol droplets (< 50 μm) for direct collection on insects. However, the smallest droplets are more prone to dispersal in air currents and can be lifted away from vegetation under hot convective conditions. It was for this reason that the drift spraying technique compromised on a volume median diameter of about 100 μm droplets. This avoided excessively large droplets which wasted chemical and yet had a large number of droplets below 100 μm, filtered out by the vegetation and insects provided the sprays were applied early in the day, preferably under adiabatic or inversion conditions.

Recent studies of deposits on grasshoppers have confirmed that better control was obtained with droplets with a vmd of 76 μm rather than 44 μm. (Symmons *et al.*, 1991).

Formulation

When small droplets are used, rates of evaporation can be high as both the convective and radiation heat transfer coefficients are inversely proportional to droplet size (Courshee, 1959). In consequence, relatively involatile oil-based formulations are preferred, although in agricultural work, water-based formulations have been used successfully if an involatile component is used to restrict the effect of evaporation on droplet size to prevent their shrinking too small.

Spray volume

This is determined on the basis of droplet size (d) and the number of droplets (n) required per unit area. Thus when spraying a flat surface the number of litres per hectare (Q):

$$Q = \frac{n\pi}{60} \div \frac{(100)^3}{(d)}$$

For example if you require 19 droplets of 100 μm per cm^2 you need to apply one litre per hectare. The number of droplets needed is affected by the amount of the insecticide that can be carried in individual droplets, and, taking into consideration that the carrier liquid needs to be relatively involatile, adhere to the insect surface on impaction, and spread and transfer the toxicant.

Spray concentration

With particulate suspensions it is necessary to have the appropriate concentration of particles in each droplet. Several studies have considered this in relation to the application of baculoviruses; thus, against *Panolis flammea* (Denis & Schiffermüller) (Lepidoptera: Noctuidae) Entwistle (1986) required a delivery of 10–20 polyhedral inclusion bodies (PIB) per droplet with $1.1–2.2 \times 10^{10}$ droplets per hectare. They obtained the best results with the lowest number of PIBs per droplet, but more droplets per unit area (Entwistle *et al.*, 1990).

If a practical optimum droplet size of 70–100 μm is selected, each droplet will have a volume of 180–524 picolitres. Thus if 1,000–40,000 conidia per insect are required to cause mortality of *Schistocerca gregaria* (Forskål), the concentration in the spray can be varied in relation to the number of droplets that might be expected to deposit on the locusts. Assuming 20 droplets of 500 picolitres impact, i.e. 10,000 picolitres, 20,000 conidia would need to be suspended in this volume of oil, i.e. 2×10^{12} conidia per litre. The chances of more droplets impacting directly on locusts will increase if the volume is increased, and the concentration of spores kept to a minimum. However the cost of the carrier oil and the logistics of maintaining a high work rate over the extensive areas with locusts, necessitate that the volume per hectare is between 0.5 and 2.0 l/ha and preferably about 1 l/ha.

Conclusion

Regardless of whether the application technique involves aerial, vehicle-mounted or hand-held equipment, locust control with liquid formulations is normally carried out at ultra-low volume application rates. Rotary atomizers are most commonly used for this purpose, and in order to be most effective it is important to optimize the rotational speed and physical characteristics and flow rate of the formulation, to achieve a narrow droplet spectrum of an appropriate size.

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Controlled droplet application of mycopesticides to locusts

R.P. Bateman

International Institute of Biological Control.

Abstract

Oil-based ULV formulations containing conidia of *Metarhizium flavoviride* have been prepared which are compatible with equipment for controlled droplet application and are toxic to *Schistocerca gregaria*. The numerical aspects of mycoinsecticide sprays are discussed and compared with the activity of chemical pesticides.

Introduction

Application of entomopathogenic fungus spores has in the past usually involved hydraulic spraying techniques. These have the advantages of versatility and familiarity, and aqueous media have often been selected in the belief that a humid environment is required for the survival and germination of fungi. Prior *et al.* (1988) demonstrated that *Beauveria* conidiospore suspensions applied to *Pantorhytes plutus* (Oberthür) (Coleoptera: Curculionidae) had lower LD_{50} values when formulated in oil than when they were water-based. The IIBC group in the Locust & Grasshopper Biological Control Programme (LGBCP) has subsequently established that locusts can become infected by topical oil-based applications even under dry conditions. However horizontal transmission is believed to be unlikely without a humid environment, therefore good spray coverage of each insect is necessary. Maximization of coverage at low volume application rates of spray mixture essentially requires the production of large numbers of small droplets – however not so small that they do not deposit on their

target (Matthews, these proceedings). This paper gives an outline of the approach taken to formulation and application in our research, and describes some initial findings.

Formulation

It is relatively straightforward to prepare oil-based ultra-low volume (ULV) formulations containing suspensions of conidia in the deuteromycete genera *Beauveria* and *Metarhizium*. Their lipophilic cuticles enable conidia to suspend easily after vigorous agitation of suitable carrier oils poured over the sporulating culture. Oils are preferred in ULV formulations since evaporation of the carrier liquid must be minimized when using small droplets, especially under dry conditions. Oils also readily stick and spread on insect exocuticles, and may assist in transport of conidia to the more susceptible intersegmental membranes and subsequent hyphal penetration (Prior *et al.*, 1988).

Wrigley (1973) gives a useful review of the properties required of mineral oils for ULV formulation. Special emphasis has been placed on substances (usually vegetable oils) that are readily available in tropical countries, and early work in this project involved their screening for toxicity to conidia. Vegetable oils have a high viscosity which must be adjusted by mixing with light paraffins so that satisfactory flow characteristics are obtained for existing equipment. The restrictor-airbleed combination for the Micron 'Micro Ulva' has been used in these tests; it should be relatively easy to 'scale up' for larger equipment used in acridid control.

Bioassays using drops placed under the posterior lip of locust pronota have been used for initial screening. Volumes of 2 µl are satisfactory, although this can be reduced to 500 nl with some increase in experimental variability. Even the latter is still several orders of magnitude larger than the volume of typical insecticide spray droplets. Preliminary bioassays have indicated that the *Metarhizium flavoviride* W. Gams & J. Rozsypal strain IMI 330189 has an LD_{50} of approximately 2×10^4 spores/adult *Schistocerca gregaria* (Forskål) after 5 days, and 10^3 spores after 8 days. At approximately 18 pg per spore, this tentative five day mortality appears to compare well with most other insecticides used against locusts (Table 1).

Using the LD_{50} measurements given above, a formulation containing 10^9 spores/ml would have a theoretical 5 day toxicity coefficient of 24 million*, compared with 5.5–10.9 million for dieldrin at field rates (MacCuaig & Watts, 1963). Toxicity coefficients based on LD_{50} data may

*Estimated number of insects which could be killed by uniformly dosing 1 l of spray formulation (half number of LD_{50}s/l).

Table 1. Contact toxicity of insecticides to adult locusts (mostly *S. gregaria*; from MacCuaig, 1983).

Insecticide	Type	LD_{50} µg/g	LD_{99} µg/g	Rate (if not LD_{99})	Time for action (>90% mortality)
Bendiocarb	ca	1.0– 2.1	1.4– 3.8		22 h
Carbaryl	ca	25–>100	69–>100		8–18 h
Chlorpyrifos	op	3.0– 3.2	6.2	5µg/g	3 d (100%)
Deltamethrin	py	0.5	13.2		5 d
DDVP	op	1.7– 8.7	6.3–27		<1 d
Diazinon	op	3.4– 6.5	7.9–17.8		3½ d
Dieldrin	oc	3.7– 6.9	8.6–25		7–21 d
Fenitrothion	op	3.7– 8.4	9.1–35		1–6 d
γ-HCH	oc	5.5–14.5	13–30		5–10 d
Metarhizium flavoviride IMI 330189	**fp**	**≈0.2**	**≈1.1**	**LD_{95}**	**<6 d**
Lambdacyhalothrin	py	0.4– 0.6	0.8– 1.9	LD_{95}	<24 h*
Malathion	op	25–38	73–110		3–9 d
Propoxpur	ca	1.0– 2.6	4– 8		5 h

Key: ca – carbamate; fp – fungal pathogen; oc – organochlorine; op – organophosphate; py – pyrethroid;
* – data for lambdacyhalothrin using *Locusta migratoria migratorioides* (Reiche & Fairmaire) which is often slightly more susceptible to insecticides than *S. gregaria* (from Alcantra Santos, 1987).

be misleading however, since the slope of the dose response curve for fungus pathogens is often much shallower than for that of chemical pesticides. The data in Table 1 show 2–3 times differences between LD_{50} and LD_{99} for chemical insecticides. In the test described above the difference was six-fold, however Lai *et al.* (1982) measured 12–62 times differences with various fungus strains active against termites, and Prior *et al.* (1988) calculated LD_{95} values that were 3–4 orders of magnitude higher than the LD_{50}s.

Controlled droplet application (CDA)

Atomizers that can produce monodisperse (uniformly sized) sprays include rotating discs, cups and cages. The centrifugal force acting on a liquid fed to the centre of the rotor causes relatively uniform disintegration at the periphery under suitable operating conditions. When spinning discs are used for applying insecticides, the usual form of atomization is via the formation and break-up of ligaments. One way of defining CDA is that a high proportion (80% or more) of the spray liquid volume should be atomized within limits of a required size band (illustrated in Fig. 1). 50–100 µm (65–254 pl) is considered a range appropriate for ULV insecticide spraying, and represents an eight-fold dose variation between the limits.

Frequently the number distribution of sprays produced by ligament disintegration has been shown to be bimodal – this is confirmed by studies using high speed photography, where the ligaments break up into principal and 'satellite' droplets. Droplet size may be adjusted most readily by changing the disc speed; the nature of the formulation itself and the flow rate of liquid has a smaller effect, however large variations in droplet size take place under conditions where transitions may occur between single droplet and ligament formation.

The 'Malvern' laser particle size analyser has been used to check that the atomization of formulations is suitable for ULV application (i.e. essentially similar to the atomization of existing ULV formulation with commonly used equipment). Fig. 1 shows the distribution of spray droplets produced with a spore formulation atomized by a spinning disc sprayer of the type used in early field trials. The average droplet volume (ADV)* is nearly 90 pl, but the distribution is bimodal with two approximately equal modes by droplet *number*; these represent volumes of approximately 10

*The volume in pl represented by the volume average diameter (VAD) or $D_{(1,0)}$. It is an estimate of the:

$$\frac{\text{Total volume of spray}}{\text{Total number of droplets}}$$ of the sample measured.

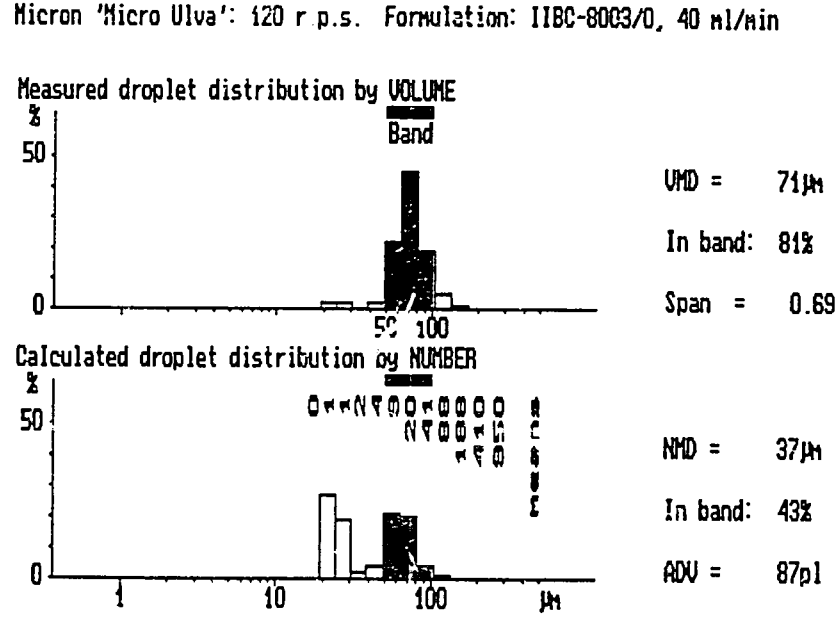

Figure 1. An example of droplet size measurement with a Malvern 2,600 particle size analyser. 'Means' indicate predicted average number of spores/droplet in each size class, for a formulation containing 10^9 spores/ml.

and 200 pl respectively. The computer programme used to illustrate these measurements also displays the theoretical number of suspended particles in each size class (in this case representing spores at a relatively low concentration for ULV spraying). It has of course been assumed that spore numbers are proportional to the volume of each droplet; this may not be the case, and is the subject of current research.

It is the larger droplets however that constitute by far the greatest *volume* of biopesticide in a spray, and droplets in this mode are also more likely to impact on stationary acridids, vegetation, and artificial targets used in field trials. At ≈ 200 pl (70+ µm diam) such droplets should typically contain approximately 200 spores at 10^9 spores/ml. Thus, 105 droplets of 200 pl on each insect might be expected to achieve a 50% mortality in 5 days with a formulation containing 10^9 spores/ml; to achieve the same result in 8 days would require only about 6 droplets.

There has been evidence that contact insecticides applied as small (≈ 20 µm) droplets are intrinsically more insecticidal for a given dose than larger ones (e.g. Himel, 1969), however MacCuaig (1962) found no significant differences in organochlorine insecticide toxicity to adult locusts with

droplets ranging from 68–250 μm. Much depends on the precise nature of the acridid target: Symmons *et al.* (1991) achieved better control of grasshoppers in the field with bendiocarb sprays of volume median diameter (VMD) ≈ 76 μm, than with a VMD of ≈ 44 μm. This is clearly a complex subject, and constitutes another line of research for the LGBCP, both in the field and using an experimental track sprayer in the laboratory.

Acknowledgements

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Development of a novel field bioassay technique for assessing mycoinsecticide ULV formulations

R.P. Bateman[1], I. Godonou[2], D. Kpindu[2], C.J. Lomer[1] & A. Paraïso[2]

[1]*International Institute of Biological Control.* [2]*IITA Biological Control Programme.*

Abstract

An experimental design is presented which allows close simulation of field conditions while retaining a degree of control more typical of laboratory situations. Oil-based ULV sprays containing conidia of *Metarhizium flavoviride* W. Gams & J. Rozsypal have readily killed locusts at humidities lower than previously considered necessary for infection by fungus pathogens.

Introduction

Different application strategies are likely to be required for the various locust and grasshopper biological control problems, but most techniques involve ULV spraying with rotary atomizers (Bateman, these proceedings). Small-scale grasshopper control may be carried out using hand-held spinning disc sprayers such as the Micron Micro-Ulva or the Berthoud C8. Larger-scale treatments for application to several hectares may be carried out with the Micron X15, a stacked array of 88 mm spinning discs mounted on a vehicle, and ultimately, aerial application using rotary cage atomizers could be envisaged for treatment of areas of greater than 10 ha.

The number of larger droplets impacting directly on insects in the field typically spans orders of magnitude, but is lower than numbers that can easily be achieved in the laboratory. This has been revealed in pilot studies using a fluorescent tracer dye (Staniland, 1959) mixed with a pyrethoid, then counting droplets on the cuticle of dead insects recovered after

spraying. It is possible that an important mode of entry for conventional insecticides might be via the smaller droplets or secondary pick-up from vegetation. The work of Symmons *et al.* (1991) suggests, however, that hand-held spinning disc sprayers should be adjusted to produce medium-sized rather than small droplets to achieve optimal performance.

Before carrying out full scale field trials, a step by step approach has been taken to the development of spray application, as recommended by Matthews (1983). This paper describes a novel technique which has been developed to spray acridid targets under controlled conditions, with droplet numbers commensurate with those encountered following normal field applications.

Materials and methods

Figure 1 is a diagram of treatment arenas used in these tests. An area larger than 50 × 50 m was mown and sixteen 3 × 3 m sampling zones were cleared of all vegetation, in four lines situated at 5, 10, 20, and 30 m downwind of a marked spray path. Four sets of samples were taken during each test, consisting of columns of sampling zones positioned parallel to the

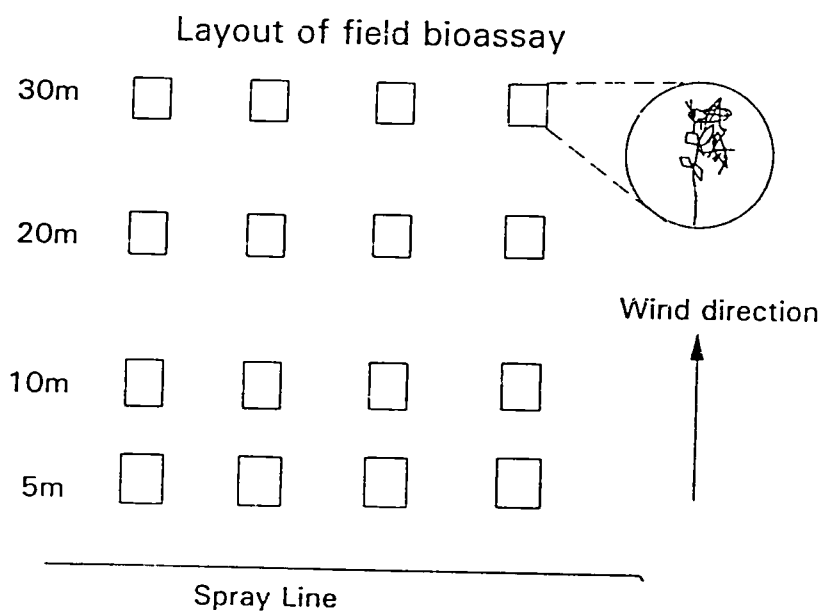

Figure 1. Layout of arena trial.

prevailing wind. Vegetation was placed in the centre of each arena to attract the locusts; in the test illustrated cassava leaves sprayed with sugar solution were used. 20 newly fledged *Schistocerca gregaria* (Forskål) adults were released onto the plants, and more than half of these could usually be recaptured immediately after spraying; locusts are usually highly active, but can be restrained using paper clips to hold their wings together. A sampling post was also sited in each arena, and droplet deposits were assessed using oil-sensitive cards pinned to it.

This design was developed after analysis of swath pattern data (Figs 2 and 3) which has also proved useful for determining practical swath widths with different application techniques. The method was first developed by Picken (in Heinrichs *et al.*, 1981) and has been modified by Bateman (1989) with faster ways of assessing and analysing droplet data. There is an initial peak in deposit, the position of which is dependent on droplet size, emission height, wind-speed, air turbulence and formulation characteristics (Johnstone, 1991). After this there is a progressive diminution of droplet numbers deposited per unit area, with increasing distance downwind of a spray line.

The spray formulations consisted of spores of *Metarhizium flavoviride*

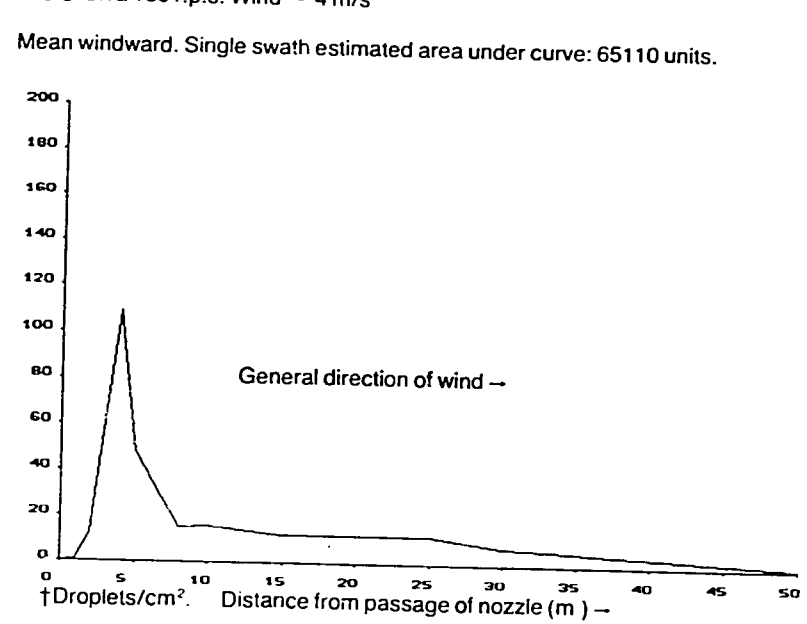

Figure 2. Droplet deposits down-wind: single swath.

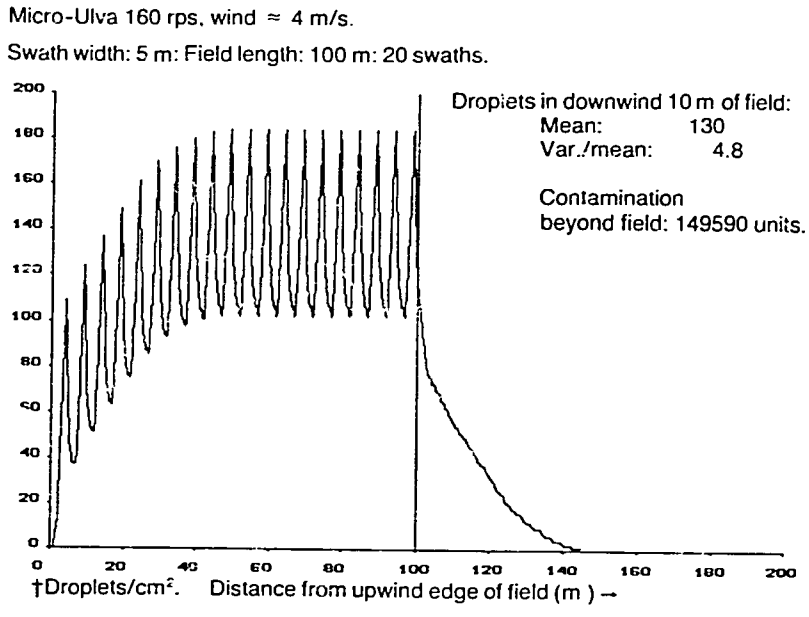

Figure 3. Incremental droplet deposition: computer simulation, from data in Fig. 2.

W. Gams & J. Rozsypal strain IMI 330189, grown on Molisch's agar and suspended in a mixture of raw groundnut oil and kerosene. Spore concentration was determined with a Neubauer haemocytometer; it was often necessary to increase spore concentration by allowing the suspension to stand and pouring off excess oil. Before spraying the spore formulation, a similar oil-based mixture containing Saturn Yellow fluorescent tracer was applied to the arena, with locusts placed along one column only (i.e. 4 sampling zones, at each distance from the spray line).

After spraying, the locusts were returned to a different rearing room from the original cultures for evaluation, since mortality can be very high with cages left in the field (J. Langewald, pers. comm.). Dead insects were placed in boxes containing moistened paper and were checked for signs of fungus sporulation.

Results

The results of two tests that were successfully carried out in Cotonou are summarized in Table 1; two others were less conclusive, in the one because

Table 1. Results of two arena tests carried out on 7/2/91 and 4/3/91 with *Schistocerca gregaria* at IITA, Cotonou.

	Formulation							Sampling position					
Test	Temp (°C)	RH (%)	Wind m/s	Spores /ml	Rates of emission[1]	% germination after spraying	Results	5m	10m	20m	30m	Control	l.s.d.
A	31	73	2–4	5.9×10^{6}	2.5 ml/m	62%	**10 days:**						
							% mortality	44	48	29	32	12	
							% sporulation	28	15	10	0	0	
							18 days:						
							% mortality	81	85	52	44	29	
							% sporulation	56	22	26	7	3	
							Mean numbers of droplets counted on:						
							Oil sensitive cards	161	101	44	32		
							(numbers/cm²) s.e.	45	12	7	4		
							insects sprayed in control (total counts):						
							head	7	7	4	1		
							thorax	26	25	18	7		
							abdomen	15	15	6	2		
							wings	44	37	33	8		
							legs, antennae	21	25	17	5		
B	32	80	4–5	1.5×10^{9}	2.3 ml/m	85%	**10 days:**						
							% mortality	87**	59	58	24	29	33
							% sporulation	48***	22*	20*	7	0	17
							18 days:						
							% mortality	95***	83**	73*	48	36	27
							% sporulation	55**	42**	29	28	3	27

1: total estimated volume (ml) emitted per 1 m of spray path.
Asterisks indicate significant differences between sampling positions and controls (*: $P < 0.05$; **: $P < 0.01$; ***: $P < 0.001$).

of premature spore germination in the formulation, and in the other due to excessive parasitism of locust stocks with *Mulamoeba*. A consistent pattern of mortality, decreasing in the order of 5, 10, 20 and 30 m downwind is usually observed. Detailed results of one of these tests are given in Fig. 4, which shows the total mean mortality at the four downwind sampling positions over a three week period after spraying. Figure 5 shows the proportion of insects for which infection was confirmed by fungus sporulation.

Discussion

The experimental layout is designed to provide a variety of mean droplet numbers at sampling stations, from which it should be possible to correlate mortality with numbers of droplet impactions. The distances from the spray line have no particular significance in themselves, apart from providing an ordinal ranking of probability of spray deposit levels. The droplet coverage on locusts can be evaluated with ultra-violet lamps.

Winds in Cotonou are relatively consistent in speed and direction and therefore it is easy to lay out tests ahead of spraying. It may be less easy to carry out similar experiments elsewhere since such consistency is exceptional away from coastal regions. Unreliable winds are a common problem

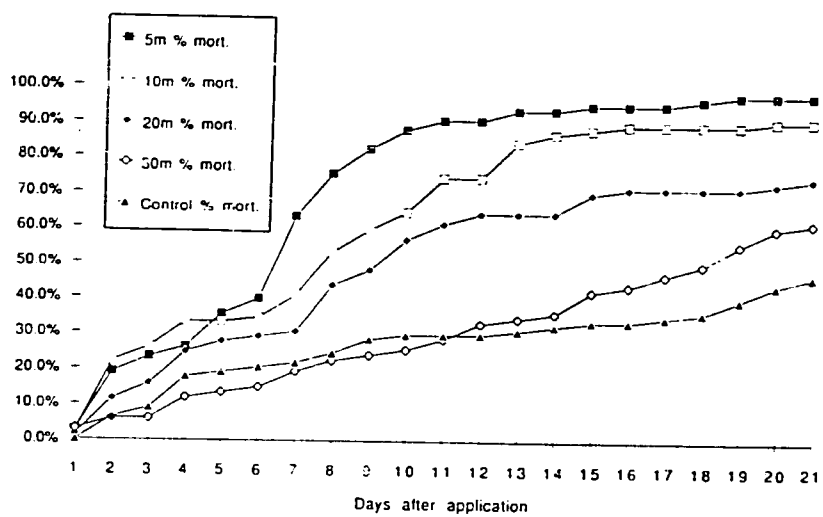

Figure 4. Results of second arena test on *Schistocerca gregaria*: *Metarhizium flavoviride* spores (@ 10^{11} spores/ml).

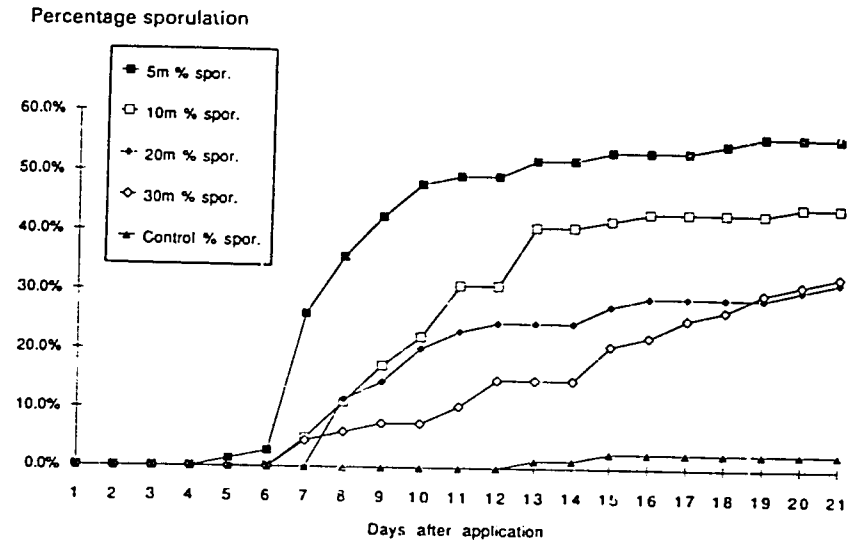

Figure 5. Same experiment as Figure 4: percentage of insects with positive identification of sporulation.

during tests and spray operations that rely on wind drift; this problem has been inadequately addressed to date by research and extension personnel.

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Equipment

'Micro-Ulva' and 'X15' atomizers obtainable from:
Micron Sprayers Ltd.,
Three Mills,
Bromyard,
Herefordshire,
HR7 4HU, UK.

Berthoud 'C8' obtainable from Technoma S.A.
54, Rue Marcel Paul,
B.P. 195 F 51206,
Epernay, FRANCE.

Panel discussion: precis

Replying to a question by Mr Abdalla, Captain Kitenda said that fenitrothion was the pesticide used which gave an unusually delayed effect against immature adult desert locusts during operations in 1988 in Sudan. Dr Auld commented on the cost of biological agents in Australia.

Dr Prior asked how bacterial contamination is avoided in the Chinese method for mass production of *Beauveria* in trenches and if the method had been attempted with *Metarhizium*. Dr Roberts stated that the *Beauveria* isolate used is aggressive and rapidly colonizes substrate material, thus circumventing the need for anti-bacterial compounds. The method has been repeated in the USA but not with *Metarhizium*.

Dr Cardwell wondered what area of rangeland had been treated in the USA with *Nosema locustae* and whether it was persistent. Dr Evans said that 1.2 million hectares have been treated with the bait formulation, and persistence varied from none to confirmed reports of seven years. Some ranchers were adding carbaryl to extend residual activity.

Dr Zelazny asked whether fungal spores are inactivated at high temperatures of around 35 to 45°C and if human allergies were a problem during the production and application of fungal spores. Dr Goettel responded by saying that although conidia are unlikely to be killed, germination and infection could be inhibited. Production, formulation and application technologies should be developed which minimize the possible risks of human allergies.

Section V

Biology, Ecology, Field Experimentation and Environmental Impact

Introduction: biology, ecology, field experimentation and environmental impact

D.L. Johnson

Agriculture Canada Research Station, Lethbridge, Alberta.

Abstract

Investigations into the effects of biological agents on target organisms under field conditions, and also their impact on the environment, can only be carried out effectively if due attention is paid to precise experimental design and methodology. Many biological agents are slower-acting and have less obvious effects than chemical pesticides and particular attention must be paid to s[illegible]ne procedures if their full impact is to be measured. Plot sizes will va[illegible]ding to target mobility, the variable under investigation and the time scale of the experiment, but due attention must always be paid to established principles of statistical design. Methods which could play a greater role in analysis of experimental and observational data are discussed and particular attention is given to analysis of covariance and to competing risk theory. Principles and techniques discussed in the paper are illustrated by reference to two field investigations on the use of pathogens for locust and grasshopper control: a field trial on the use of *Nosema locustae* Canning for control of Canadian grasshoppers and a laboratory trial on the interaction of two *Nosema* spp. infecting the migratory locust, *Locusta migratoria migratorioides* Reiche & Fairmaire.

Introduction

Although the topic of this technical session may at first seem quite wide-ranging, the organizers have selected four areas which are indeed closely linked. Field experimentation is the required proving ground of any of the results of our laboratory experiments or computer models concerned with the biological agents that have been discussed during this workshop.

Potential biological control agents must pass repeated and carefully designed testing before researchers proceed to development of the agent for applied use, and during this period numerous questions arise, while some of the earlier questions are answered. One of the crucial questions waiting for any new agent of insect control that reaches the field testing stage is whether any adverse environmental impact is likely, and ultimately this question also must be examined in the field. Thus, biology and ecology are the matrix within which effectiveness and non-target safety must eventually be studied, and the search for improved efficacy and safety are linked by a mutual reliance on precise experimental design and methodology.

Part I: design

After reviewing the published literature on the agents that could provide successful biological control of grasshoppers and locusts (examples include *Entomophaga grylli* (Fresenius) Batko, *Beauveria bassiana* (Balsamo) Vuillemin, other fungi, *Nosema locustae* Canning, *Farinocystis* sp., parasitic Diptera and mites), it is clear that although field experiments must be designed to suit the special attributes of each agent and its relationship to the host target, some common attributes of field experimentation are required for valid hypothesis testing and decision making. The impact of biological control is often not as clear as in the case of field experimentation with chemical insecticides, nor are biological control studies limited to measurement of mortality alone. Therefore, there exists in this area of research an even greater need for correct experimental design and for useful application of powerful new methods of analysis. Before reviewing past results in the panel discussion, or attempting to standardize our research protocols, it is worth our time to review proven practices and new developments, and consider applications. Speakers have been asked to draw on their own experience, so I will use some examples with which I am familiar.

The basic steps in field experimentation are design, execution, analysis and interpretation. Each of the attributes of the overall design is based on or composed of considerations regarding the questions and measurements of interest (Table 1). Our field results from planned experiments in biological control of grasshoppers should be weighed in the context of these attributes.

When testing biological control agents, for example fungal spores applied as a microbial insecticide against grasshoppers, ecological attributes of both the target and the pathogen must be considered in the context of the particular hypotheses to be examined. The particular attributes of

Table 1. Fundamental considerations in field experimentation.

Hypotheses
— Include both research hypotheses and those required for statistical tests
— Determine the measured variables and suggest design attributes

Experimental design
— Demands for repeatability
— Restrictions on randomization
— Choice of variables to be controlled and monitored
— Method of measurement and sampling

Concomitant and nuisance variables

Experimental and sampling error

Analyses and tests
— Interpretation, may lead to revision of hypotheses; new subsets take precedence
— Lead to redesign and implementation of subsequent experiments
— Communication of results

acceptable field trials depend on the questions being asked. For example, it is quite valid to conduct a microbial treatment in cages of 1 m^2 when the questions of interest concern interaction of synergistic agents with the spores over a short period, or when precise measurements of reproduction or feeding are to be made, or when only small amounts of inoculum are available for a first outdoor test of pathogenicity (for example, cage trials – Johnson *et al.*, 1988). It is, however, also valid to conduct treatments with an agent in larger plots, each 10 to 20 ha, for timing trials that presume limited movement of the target insect over less than one season (e.g., Johnson, 1989c), and even larger plots are required in cases in which problems with movement are anticipated (e.g., the fifteen 200 ha plots used in Mali in 1989 by Van der Paauw *et al.*, 1990). Very large plots, such as in the treatment of plots of thousands of hectares per plot by Henry & Onsager (1982) are required to ask other, equally valid but different questions. There is no single plot size, application method, or even sampling method that is always right (or always wrong) for a particular biocontrol agent or target pest species, but there are of course standards of design that are universal requirements, such as the employment of replication, randomization, adequate sample sizes, and well-tested assay methods. Even enormous plot size does not guarantee a useful test if the need for replication and randomization are ignored.

Sometimes field experiments must appear to break the rules in order to test them. For example, it has been generally accepted that the microsporidan *Nosema locustae* should be targeted at the peak modal density of the third instar. A recent field test in Alberta of this hypothesis dispelled

this notion, and will serve here to illustrate some of the basic principles involved in field experimentation involving biological control of grasshoppers.

An experimental example: the effects of timing and frequency of application of *Nosema locustae* on the infection rate and activity of grasshoppers

Introduction

The ability of *Nosema locustae* to debilitate grasshoppers and locusts by attacking the fat body and other internal organs has suggested that, although it is slow-acting, this entomopathogen may have application as an environmentally safe, long-term management tool for grasshopper control (Henry, 1971, 1972).

Although the detrimental effects of *N. locustae* on grasshopper growth and survival can be demonstrated easily in laboratory experiments, the results of field experiments designed to measure its effectiveness vary widely. Field results have varied from no discernable impact up to 60–70% mortality with infection of 95–100% of the surviving grasshoppers (although the experiment in which this best result was observed (Ewen & Mukerji, 1980) was unreplicated, having only one plot of each treatment, and assessment of the change in population density was not based on actual counts of grasshopper density). More typical results fall between these two extremes, attaining 30–50% grasshopper mortality caused by *N. locustae*, and infection of 20–50% of the survivors. In atypical situations, such as application against roadside grasshopper populations, only about 10% are infected (Johnson & Henry, 1987).

Because the primary goal of both experimental and practical field application of *N. locustae* has been to reduce the number of grasshoppers in the treated area, the more susceptible third and fourth nymphal instars are targeted (Henry *et al.*, 1973; Henry & Onsager, 1982). Late-season application is considered inadequate, or at best inferior to earlier application, because the larger and more robust adults are less likely to succumb to the disease. Application too late in the season is a common explanation given for poor field results. However, the nonlethal symptoms of infection include significant suppression of feeding (Johnson & Pavlikova, 1986) and of reproduction. It is also reasonable to assume that higher rates of infection of the adults would increase the rate of transmission of the pathogen to the next generation. In the interest of maximizing the percentage of the grasshopper population successfully infected, the effects of the timing and frequency of *N. locustae* application were examined without the constraint of following the methods aimed at attaining maximum grasshopper mortality. Because of the controversy surrounding demonstrations of efficacy, the comparisons were made as part of a rigorous field experiment.

Methods

The *N. locustae* bait spores used in this experiment were produced and formulated on wheat bran as described by Johnson (1989a). The spore concentration of the formulated bait was 1.3×10^9 spores/kg bait (wet weight).

Four blocks of native and improved grass pasture near Taber, Alberta, Canada, were each divided into four 16 ha plots. Each plot measured 200 × 800 m. This was considered to be a minimum size for a 9 week trial. A complete set of the following four treatments was randomly assigned to the plots in each block: early-season bait application (June, when younger instars 2–4 predominate), late-season application (July, when a majority of grasshoppers are in the adult stage), double application (both early and late application to the same plot), and no bait application (control). The treated plots received wheat bran bait containing 3.3×10^9 spores/ha at each application in 2.5 kg of wheat bran bait/ha.

Measured variables were: the infection rate, defined as the percentage of grasshoppers collected during the experiment that were found to contain *N. locustae* spores; and the grasshopper population density, estimated by making counts of grasshoppers observed in 320 permanent quadrats and by collection of grasshoppers with sweepnets. The two methods were employed because under typical field conditions the first provides an unbiased estimate of population density of active and lethargic insects alike, while the latter method depends on grasshopper condition and activity (Johnson *et al.*, 1986).

The $\log_e$-transformed count data and the number of sweeps per 100 grasshoppers were analysed separately for each post-treatment sampling date, using analysis of covariance. Since the quadrat positions did not change during the experiment, the pre-treatment observation (week 0) was the concomitant variable. Treatment effects were tested with experimental error (block by treatment interaction), experimental error was tested with subplot error, and subplot error was tested with sampling error among quadrats. Orthogonal contrasts were used to compare the following treatment combinations: early vs late treatment, single vs. double treatment, and treated vs. untreated.

Results and discussion

Grasshopper population mean density in the plots at the time of treatment ranged from 5.0 to 7.5/0.25 m^2. Table 2 indicates how the early and late treatments differed in age structure. The results were surprising: although

Table 2. Grasshopper species and age structure at the time of application of *N. locustae* bait in the timing experiment. (The total number collected and the percentage of each species is shown.)

Species	% Adult and species sample size			
	Early application[a]		Late application[b]	
Melanoplus infantilis Scudder	1.1	2584	83.1	2468
Camnula pellucida (Scudder)	6.6	1341	97.0	1914
Melanoplus sanguinipes (Fabricius)	3.5	935	69.5	902
Melanoplus packardii Scudder	3.2	155	92.2	192
Ageneotettix deorum (Scudder)	0.0	107	89.3	177
Spharagemon campestris (McNeill)	0.0	85	58.3	175
Trachyrhachys kiowa (Thomas)	0.0	80	49.0	98
Rare species (combined)	1.0	102	47.3	188
All species	2.9	5389	83.6	6114

[a] 25 June 1986.
[b] 16 July 1986.

the mortality rates did not differ, the later application, when older grasshoppers predominated, resulted in significantly higher infections.

Although the infection rate that resulted from the early application of *N. locustae* bait was initially higher than that caused by the late application, the late application of spores infected more individuals by the end of the experiment ($\chi^2 = 7.97$, $P<0.01$). The effect occurred consistently: later treatment resulted in a higher rate of infection at all four block locations (Table 3). The degree of infection also differed between the early and the late treatments. The early season infections were not more advanced or dense.

On no date did early application result in a greater or lesser decline in grasshoppers than resulted from later application (up to 8 weeks, $P<0.2$). At 4 weeks, the overall adjusted mortality (Abbott, 1925) in the treated plots was 39% or 8%, 44-, 35- and 14% in blocks 1–4, respectively. This is typical of the range of response to *Nosema* treatment. We can conclude that timing is not as critical a factor as was believed, relative to other variables affecting virulence and efficacy.

The number of sweeps required to collect a fixed number of grasshoppers increased significantly in the plots treated with *N. locustae*. The estimates of the reduction in density from the quadrat samples were less than the estimates based on sweepnet samples. The greater sweeping effort required in the plots treated with *N. locustae* reflects symptoms of the disease which are expressed as reduction in activity or a change in position of the grasshoppers. This second result indicates the importance of careful selection of sampling methods.

Table 3. Repeatability of the greater infection rate resulting from late treatment. The values are percentages of grasshoppers infected 9 weeks after the early and 6 weeks after the late bait application (n = 240 grasshoppers assayed per block) (Johnson, 1989a).

Timing	Block 1	Block 2	Block 3	Block 4
Untreated	6.7	8.3	3.3	5.0
Early	16.7	33.3	23.3	18.3
Late	46.7	35.0	30.0	26.7
Early & Late	36.7	43.3	41.7	33.3

Part II: results and analysis

Before we look at the results of more field trials, it is helpful to list and review some accepted and new methods that are useful in interpretation. Data that require such interpretation include:

Measured variables

- Population dynamics of target
- Survival and activity of agent
- Direct and indirect effects
- Primary and secondary impact

Impact

- Primary
  - infection
  - predation
  - parasitism
- Secondary
  - crop protection
  - synergism with other agents
  - target reproduction
  - short- vs. long-term impact

Some useful methods for tests on trials of entomopathogens and the applications of results of these trials include the following:

- Analysis of covariance
- Probits, logits, logistic regression
- Discrete multivariate analysis counts

- Simulation and other non-statistical modelling
- Competing risks theory
- Spatial and geographic analysis
- Expert systems

This list is a mix of often overlooked standard methods, and some new methods that would benefit field tests of microbial pesticides. The application of analysis of covariance in field experimentation on biological control of locusts and grasshoppers is an obvious but neglected tool that has been mentioned in the preceding example. If the sampling sites (quadrats, for example) are uniquely identified from start to finish in assessments of impacts of a treatment on grasshopper population density, the pre-treatment density can be profitably used as a covariate. 'Profitably' means that a sufficient portion of the variability is accounted for by the initial densities to make up for the loss of the one degree of freedom required for inclusion of the pre-treatment density in the model. The user may also be alerted to different degrees of effect under conditions of high and low grasshopper density, because analysis of covariance adjusts the result on the scale of the initial number. Adjustment of the post-treatment counts by the pre-treatment densities is often necessary, but is less preferred because no variation (SS) is accounted for in this way.

Probit-type models can be of use in cases in which we find a dose-mortality relationship (LD), or in which estimation of the rate of accumulation of mortality is important (LT), although these methods are more generally applicable to carefully controlled laboratory trials. Discrete multivariate methods (Bishop *et al.*, 1980) are of value, because our data often consist of counts for which we wish to compare and assess interactions, to which this method of analysis is well-suited. Computer simulation, usually based on difference equations and numerical integration, is a powerful method of formalizing our conception of the system and determining how the system might behave.

A less well-known method that is useful in this particular area of research is the application of competing risks theory. This is a body of statistical theory which seems to have been overlooked by entomologists but which may have important applications in insect experimentation and population modelling. The theory is not new, but has been used mainly by demographers and medical statisticians concerned with analysis of data involving death due to one of several possible causes. Competing risks theory deals with situations in which there is interest in the failure (or exit) times of individuals, where the subjects are susceptible to two or more causes of failure, and where the failure occurs over time. Questions for which competing risks theory is designed to provide solutions include:

1. inference on the effects of treatments or other covariables on specific types of failure

2. inference on the ways in which the failure types are related under specific conditions
3. estimation of failure rates for some causes if some or all other causes were eliminated.

You will recognize the last point as a variation of a question sometimes asked during the interpretation of the often murky results of biological control field experiments: 'considering the circumstances, do we have something here or don't we?'.

Competing risks theory is useful in analysing the results of biological control trials because the models used in analysis of this type of experiment, where the impact on the insects may take more time than mortality caused by insecticide, are based on stage-frequency data. The models or concepts are built around the idea that upon entering a growth stage, insects spend a certain amount of time in a stage and then exit from it either by dying or maturing to the next growth stage. The theory allows assessment of the simultaneous action of the agent on mortality and on developmental success and rate. Explanation of the models involved and several formulations of the theory with application to insect experimentation are given by Schaalje *et al.* (1989).

An interesting example of application of the value of competing risks theory occurred in an experiment designed to determine and compare the effects of *Nosema locustae* and *Nosema cuneatum* Henry on development and mortality of African migratory locusts *Locusta migratoria migratorioides* Reiche & Fairmaire (G.B. Schaalje, D.L. Johnson & H.R. van der Vaart, unpublished data).

Information was desired not only on the rates of successful maturation as a function of inoculation rate, but also on the distributions of the maturation times.

Third instar locusts (N=720) were inoculated *per os* with spores of either *N. locustae* or *N. cuneatum* at one of the following rates: check (0 spores), 1×10^2, 1×10^3, 1×10^4, 1×10^5, or 1×10^6 per disk. After a 24 hour feeding period, the treated hoppers were transferred to individual 500 ml plastic cages for observation until reaching adulthood. Initial weight, sex (roughly equal numbers were used) and condition were recorded. They were fed daily, and dates of moulting, date of death, final weight, and level of infection were recorded.

The observed locust maturation time did not increase with increasing dosage of *N. locustae*, even though percentage mortality did increase with increased dosage. One might erroneously conclude that *N. locustae* did not affect development, but competing risks analysis and proportional hazards analysis indicated that this seemingly obvious conclusion would be incorrect. The 'force of maturation function' indicated that maturation decreased significantly as dosage increased, but that the effect had been

obscured by mortality. *N. cuneatum* appeared to have only a slight impact on maturation, but the estimated hazard functions clearly showed that maturation is severely affected by high doses of *N. cuneatum*. I will not spend more time on this particular method of analysis here, but it shows that one can be misled by looking for only the 'obvious' effects when two competing results, such as maturation and death, are measured in the same experiment.

Field experimentation on the biological control of grasshoppers may benefit from spatial and geographic analysis. New technology in this area has allowed the assessment of the role of large-scale geographic factors in determining changes in grasshopper distribution and abundance (Johnson, 1989b). The same technology allows the user to determine and map the environmental conditions that may allow a biological control agent to operate effectively, or to at least determine the regions that allow survival of a pest. An example of the latter use is the CLIMEX system developed by CSIRO Division of Entomology, Australia. Other modern geographic information systems are in use for forecasting grasshopper abundance, in Alberta since 1987, and also recently in Montana (Kemp *et al.*, 1989), and Saskatchewan, and by FAO and other agencies interested in forecasting African grasshopper and locust appearance (Cherlet, 1990). Adaptation of the software and database to include natural enemies is likely to be completed within the next few years. An example of a geographic-scale application would be to monitor the occurrence of entomopathogens over a large area, for example *Entomophaga grylli* in the Canadian Prairies (Erlandson *et al.*, 1988).

Part III: environmental impact

There have been several detailed reviews of the impact of possible microbial control agents of grasshoppers. Burges (1981) summarized the ways in which microbial pesticides may present a hazard to health, infection of non-targets including people, effects of toxins on human health, allergic reaction, carcinogenesis, and mutagenic effects. Other possible hazards include ecological impact resulting from removal of pests with complex ecological relationships, and the impact on non-target arthropods. The former is a rare but interesting possibility, but the latter is a more likely hazard, especially when applying microbial pesticides with a wide range of hosts, e.g. *Beauveria bassiana*. However, it has been noted (Prior, 1990) that even these non-specific fungi are limited by environmental restrictions and probably do not present insurmountable problems. The host range, specificity, and registration status of fungi that could affect nontarget invertebrates are reviewed in detail by Goettel *et al.* (1990).

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The *Beauveria–Melolontha* project: experiences with regard to locust and grasshopper control

S. Keller

Federal Research Station for Agronomy, Zürich.

Abstract
A project on the use of the fungus *Beauveria brongniartii* (Saccardo) Petch to control the soil inhabiting larvae of *Melolontha melolontha* Linnaeus, which has been running in Switzerland for nearly 20 years, is summarized. Parallels to the possible use of fungi for the control of locusts and grasshoppers are pointed out and discussed. Emphasis was put on strain selection, mass production, formulation, application and monitoring the trials.

Introduction

In the early 1970s a project was started at the Federal Research Station for Agronomy at Zürich to control *Melolontha melolontha* Linnaeus (Coleoptera: Scarabaeidae) with the fungus *Beauveria brongniartii* (Saccardo) Petch (Deuteromycetes: Moniliales). This pathogen was chosen mainly for the following reasons:

1. it is considered as one of the most important antagonists (Keller, 1986)
2. it proved to be the most promising candidate of all tested pathogens (Hurpin & Robert, 1972)
3. it is easy to produce
4. it is a specific pathogen of *Melolontha* spp. (Baltensweiler & Cerutti, 1986).

Biology and ecology of the host

In the research area *M. melolontha* develops in three years. The larvae live in the soil and feed on roots of nearly all cultivated plants including trees. The adults swarm mainly in May and concentrate at the borders of forests. After a feeding/egg maturation period of about 7–10 days the females fly back to the breeding sites (mainly meadows and orchards), burrow into the soil and deposit egg batches. A female may produce up to three egg batches. Development of the individuals is regionally synchronized so that swarming of adults takes place every three years.

Periodic fluctuations characterize the population dynamics. In the past, population outbreaks occurred every 9–15 generations (27–45 years) and lasted 4–5 generations. The length of the periods of low density was more flexible and lasted 4–10 generations (Keller, 1986). There are three theories to explain this fluctuation:

1. climatic conditions (Richter, 1966)
2. availability of food for the larvae (Schütte, 1976)
3. diseases (Keller, 1986).

About 40 micro-organisms and nematodes are reported to be associated with *Melolontha* spp. About 10 can be considered as primary pathogens. Some are very rare (e.g. viruses), others occur regionally (e.g. *Rickettsiella melolonthae* Krieg); fungi and protozoans seem to occur throughout the distribution area.

The use of the fungus *Beauveria brongniartii* to control *M. melolontha* in Switzerland

Strain selection and quality control

Nearly 300 strains of fungi attacking *M. melolontha* have been isolated from larvae, pupae and adults, originating from different geographic regions of Switzerland. They show a wide variation in growth patterns as well as in virulence. Also the susceptibility of larvae from different regions varies widely.

Strains used for blastospore production and aerial application were selected by virulence tests on larvae and adults. Those used for conidia/mycelium production and soil treatment were selected by virulence tests on larvae only. In all cases, re-isolates from infected hosts were used for the field trials to prevent an eventual decrease of virulence by too long cultivation on artificial media.

The use of blastospores

Blastospores were used for helicopter application to treat the swarming adults. In this method the egg depositing females are used to carry the disease into the breeding sites. Precise timing of spray application is necessary since feeding/egg maturation and incubation period last about the same length of time. Furthermore, the blastospores used in these treatments cannot be stored for more than 4 weeks. Consequently the time between the end of production procedure and application should be kept as short as possible, which may cause problems.

For the field trials we selected two to five strains pathogenic for both adults and larvae. The strains were first multiplied separately but later mixed for the final production steps which took place in a 5,500 l fermenter (Keller *et al.*, 1989). The final broth contained about 2×10^9 spores/ml.

The suspension was applied at a concentration of 4×10^8 to 2×10^9 spores/ml. Skimmed milk was added as a sticker and UV-protectant. The spray flights took place in the late afternoon and evenings to reduce inactivation by sunlight. The dosage amounted to $2–4 \times 10^{14}$ spores/ha.

The use of conidia/mycelium

The fungus was cultivated on barley kernels in autoclavable polyamide bags (Aregger, in press). After a growth time of about 4 weeks at 18–23°C the kernels were dried and cooled. At that time the fungus had completely colonized the kernels and started to produce conidia. At 2°C the material can be stored for about a year. The strain and the mode of storage strongly influence the length of storage (Aregger, in press).

The kernels colonized with *B. brongniartii* were applied with a drill machine which allowed their deposition at a soil depth of about 3–5 cm, i.e. in the habitat of the white grubs. In the soil under normal conditions the fungus grows on the kernels and produces conidia. The fungal masses on the kernels remain clearly visible for 2–5 months depending on the season. In the first two field trials under practical conditions 90–120 kg kernels/ha were applied.

Monitoring the trials

Blastospore trials

The following parameters were checked:

1. viability (germination rate) of the blastospores by samples taken immediately before spraying

2. the effect of spraying on the adults by samples taken before and after the treatments
3. the reproduction rate (ratio of the density of the larvae after the treatment to the density of the adults before the treatment)
4. the feeding damage of the adults in the generations before and after the treatment
5. population dynamics was monitored by soil samples taken each year. Samples of larvae were taken to determine the infection rate. Both samplings were started usually two years before the treatment. The efficacy is expressed as change of the population density after the treatment in comparison to the situation before.

Conidia/mycelium trials

Population density was monitored by soil samples, the first one taken before the treatment, the second one about 2–5 months after the treatment and the following ones in intervals of a year. The efficacy is expressed as above (5).

Results

The results were published in detail (Keller *et al.*, 1986, 1989; Keller, 1989, 1991). They can be summarized as follows.

Blastospore trials

Treatment of the adults resulted in mean infection rates between 63 and 96% and in a reduction of reproduction rate from 5.1 to 2.15 second instar larvae/adult in the trials of 1988. At all sampling sites the fungus established in the *M. melolontha* populations during the generation after the treatment. The disease developed epizootically in the second generation after the treatment but only at densities greater than 20 larvae/m^2. At 11 treated sites we followed the development of the populations for at least two generations. At four sites the efficacy was good (more than 80% density reduction), at three other sites it was sufficient (50–80% reduction). No effect was observed at three sites. This failure is attributed to the low population density.

In this method the fungus acts slowly and probably depends on the population density. It is therefore unsuitable for achieving rapid, overall control; it seems rather to be a method for long-term regulation of the populations, as is demonstrated by the trial carried out in 1976 (Fig. 1).

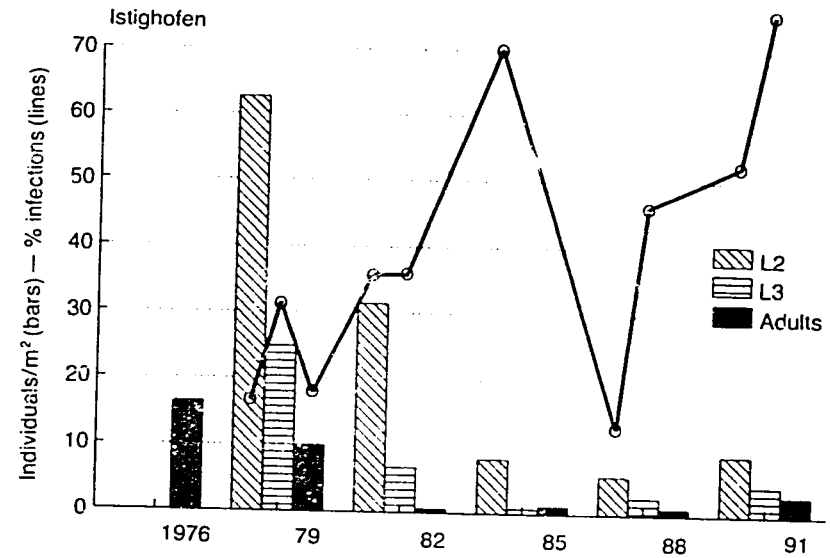

Figure 1. Development of the population density (individuals/m^2) and of the percentage of individuals succumbed to *B. brongniartii* (% infection) following the treatment of the swarming adults with blastospores in 1976. The 3 connected columns represent a generation. Infection rates are given only in years marked with a circle.

Conidia/mycelium trials

Five months after the treatment, the treated and the untreated populations did not differ remarkably in their densities but in their infection rates. The fungus needed one more year to distinctly reduce the densities (Fig. 2). The efficacy amounted to 84% in an orchard and 98% in a meadow. Control of the pest insect in the orchard was sufficient, better than with chemicals, and clearly visible by the health of the trees.

Comparisons and connections with locust and grasshopper control

Biology and ecology of the pest insects

M. melolontha spends most of its life in the soil whereas locusts and grasshoppers only have their eggs there. Both have periodic population outbreaks during which *M. melolontha* increases densities and enlarges the

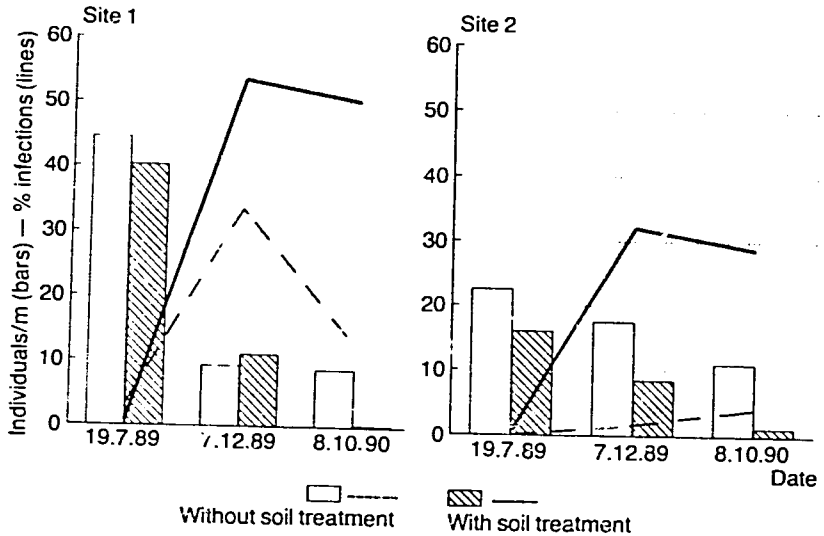

Figure 2. Development of the population densities and of the infection rates at two sites following a soil treatment carried out on 13.6.1989. Site 1: natural meadow, adults treated with blastospores by helicopter in May 1988; site 2: apple orchard with usual chemical treatment of the white grubs.

breeding areas, whereas locusts migrate. The collapse of *M. melolontha* populations is often initiated by diseases.

M. melolontha keeps its breeding sites and feeding places for generations and even over several cycles. This site-fidelity makes it easy to survey the populations and to plan eventual control measures well in advance.

Considerations on the use of fungi

B. brongniartii was selected as the control agent mainly for the following reasons, it occurs naturally and often causes epizootics, in laboratory and field trials it proved to be more virulent than other pathogens, it can be produced in large quantities on solid or inexpensive liquid media, and it is host specific.

The submersed production requires expensive equipment and experienced staff. *B. brongniartii* produces blastospores which cannot be stored for a longer period without special formulation. Other fungi (e.g. *B. bassiana* (Balsamo) Vuillemin) produce blastospores and/or conidia in liquid media. The type of the resulting spores may be influenced by the fermentation procedure. A new fermentation technique makes it possible

to produce mycelial globules, which can be dried and stored. After rehydration the mycelium produces conidia (Andersch *et al.*, 1990).

A surface production of *Beauveria* spp. and *Metarhizium anisopliae* (Metschikoff) Sorokin can be done with less expensive equipment and less experienced staff. But it requires more time, staff and space. Such a product, however, can be stored under cool and dry conditions for many months.

The strains to be used must be carefully selected. Fungal strains vary considerably in respect of pathogenicity, virulence, production patterns and other characteristics. The target insects as well may vary in their susceptibility, depending not only on age and instar, but also on population density and geographic origin. It is, therefore, important to search for the best host-pathogen combination. The fungal strains must be stored or maintained in a manner to preclude loss or reduction of virulence.

Since locusts and grasshoppers live on the soil surface and are distributed over a vast area, only an aerial treatment makes sense. Many questions remain, for example, dosage, type of fungal material, formulation (sticker, UV-protectant, hygroscopic additives, oils, baits, combinations of different pathogens), application time in respect of both climatic conditions and stage of development (individual, population), area to be treated, self-propagation, distribution of the fungus, etc.

The aims of the treatments are to define:

1. killing the animals
2. protection of crops
3. prevention of forming swarms
4. limitation (regulation) of the population growth.

The field application must be adjusted to these aims and be monitored in a way which allows conclusions about success or failure, and about why and how to improve the method.

When setting up a schedule we must be aware that many time consuming attempts have to be made and many methods and approaches have to be tested to draw final conclusions on the feasibility of the use of fungi to control locusts and/or grasshoppers.

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Biological control of grasshoppers in the Cape Verde Islands

M.L. Lobo Lima[1], J.M. Brito[1] & J.E. Henry[2]

[1]*National Institute for Agricultural Research, Cape Verde;* [2]*Montana State University.*

Abstract

Beauveria bassiana (Balsamo) Vuillemin was tested against *Oedaleus senegalensis* (Krauss) and *Diabolocatantops axillaris* (Thunberg) in Cape Verde. Formulations in mineral oil and oil-water emulsions were applied in the field to 1 ha plots and also to the vegetation in cage trials before grasshoppers were introduced. *Nosema locustae* Canning and *B. bassiana* were also tested as baits. *B. bassiana* caused a statistically significant mortality when sprayed directly on to the insects in the field but application to vegetation in cages was less effective. Infection occurred in the *N. locustae* trials but was insufficient for control. Studies with the entomopox viruses of *Oedaleus* and *Melanoplus* and a survey of pathogens in Cape Verde grasshoppers are reported.

Introduction

During the past ten years, the National Institute for Agricultural Research (INIA) in Cape Verde has been investigating integrated control of major crop pests. This research into biological control methods has concentrated on the use of predators and parasites of insect pests of irrigated crops. Research into biological control of grasshoppers began in 1989 when INIA in collaboration with Montana State University (USA) and with financial support from USAID, initiated research on grasshopper pathogens. The entomopathogens *Beauveria bassiana* (Balsamo) Vuillemin, *Nosema locustae* Canning and the entomopoxviruses OPV (*Oedaleus* entomopoxvirus) and MPV (*Melanoplus* entomopoxvirus) have been tested against

Oedaleus senegalensis (Krauss), *Diabolocatantops axillaris* (Thunberg) and other species. Also, surveys are being conducted for endemic pathogens in the native grasshopper fauna of Cape Verde.

Current research situation and prospects

Beauveria bassiana

The strain of *Beauveria bassiana* used against *Oedaleus senegalensis* in trials in Cape Verde on the island of Santiago, was provided by Mycotech Corporation (Butte, Montana, USA), which has developed production techniques for this entomopathogenic fungus and which participated in conduct of the trials. The spores of *B. bassiana* were formulated to produce a dry powder containing 4×10^{10} spores/g. Two field trials were conducted involving both general field applications and field cages and which were supervised by INIA research scientist, F. Delgado.

Field dosage evaluation

Dosages of 5×10^{12} and 2×10^{13} conidia/ha were applied by spraying 5 l/ha of an oil suspension using Micro-Ulva sprayers. For each dosage, three plots of 1 ha, distributed in three blocks, were treated. The same number of plots was used for controls. The evaluation of results was by assessing variation in population density measured with rings (Johnson *et al.*, these proceedings), and by the percentage mortality of grasshoppers collected 24 and 72 hours after treatment and held in the laboratory to observe the development of infection.

The infectivity of this *B. bassiana* strain to *O. senegalensis* was clearly demonstrated. For grasshoppers collected 24 hours after treatment, significant mortality was noted compared with the control, with infection of 30% for the highest dosage (Fig. 1). Mortality in grasshoppers collected 72 hours after was not significant (Fig. 2) although, for the higher dosage, mortality was higher than the control in all plots treated.

Estimation of the population density of *O. senegalensis* based on counts of grasshoppers in rings showed a very large variation, and accordingly, it was not possible to demonstrate significant differences between treatments and controls (Fig. 3). However, in two of the plots treated with the higher dosage, a very high reduction in the population was noted.

Formulation trials in cages

In this trial three formulations were evaluated at dosages of 5×10^{12} and 2×10^{13} conidia/ha. The formulations were as follows:

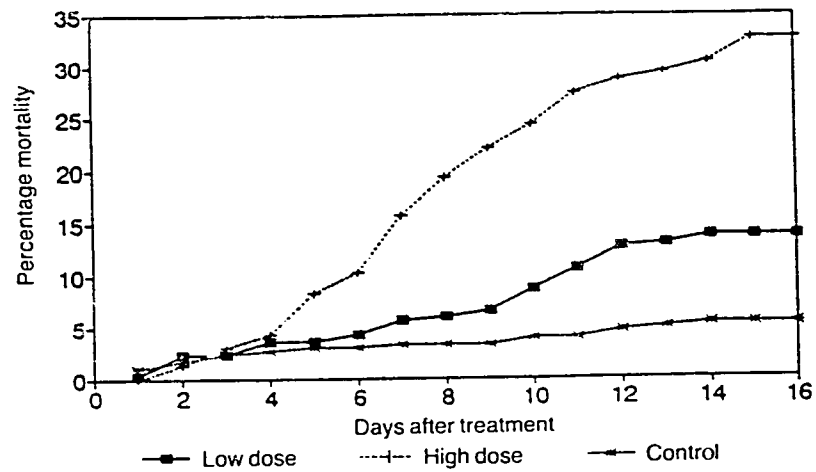

Figure 1. Mortality of grasshoppers collected from the field 24h after treatment with *Beauveria bassiana* and held in the laboratory.

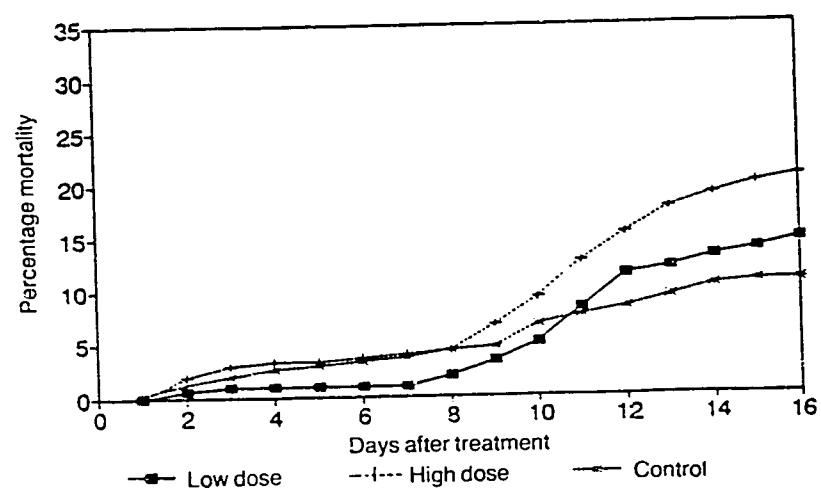

Figure 2. Mortality of grasshoppers collected from the field 72h after treatment with *Beauveria bassiana* and held in the laboratory.

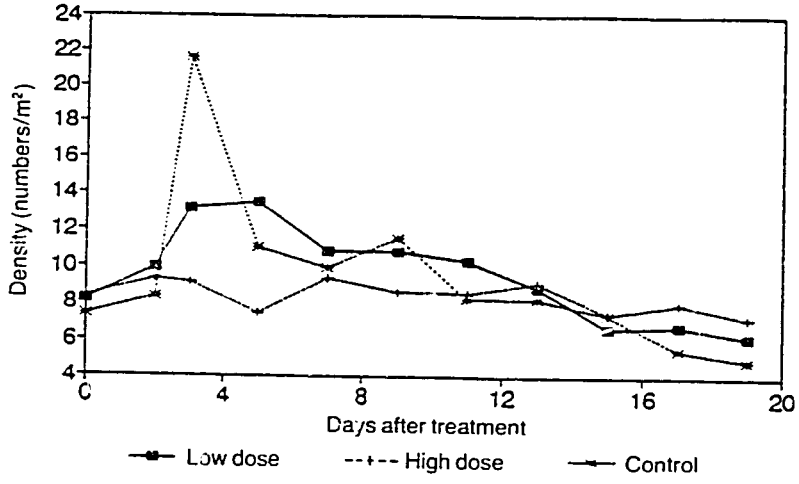

Figure 3. Population density of grasshoppers in plots treated with *Beauveria bassiana*.

1. suspension of conidia in oil
2. suspension of conidia in oil mixed with water (10:1)
3. conidia in a bran bait.

The suspensions were applied using Micro-Ulva sprayers. For the 5×10^{12} dose, 5 l/ha of liquid were applied and 10 l/ha for the 2×10^{13} dose. The bait was applied at 0.1 g and 1 g per cage. Fifty grasshoppers were introduced into each cage after treatment of the plots. Infection of the grasshoppers was by both contact and consumption of vegetation and bait. Grasshoppers were counted on each of 20 days after treatment in each cage. Dead grasshoppers were removed and placed in incubation chambers for diagnosis of the infection.

The results were based on the average number of live grasshoppers for each formulation and each dosage. There were no significant differences between treatments with conidia in oil suspension and control. However, the percentages of grasshoppers still alive at 12 and 20 days after treatment with *B. bassiana* as bait were 12 and 5%, respectively, compared to 50% still alive in control groups (Fig. 4). It was not possible to assess results for the oil and water formulation, because several cages were destroyed by animals. Both bait treatments caused significantly greater mortality than that which occurred in the control cages.

The results of these field cage trials indicated that formulation was an important factor in the use of *B. bassiana* as a bio-insecticide. The application of spores in the oil suspension to vegetation was not effective in Cape

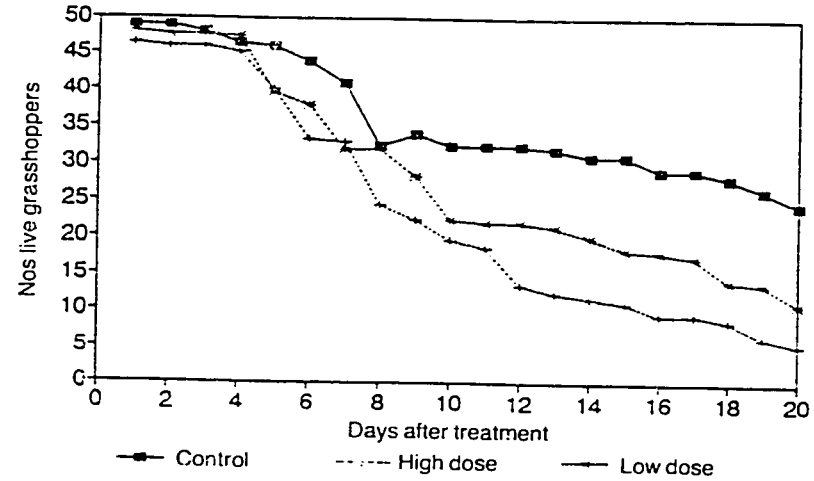

Figure 4. Results of the application of *Beauveria bassiana* as a bait.

Verde, contrary to the results obtained in Montana (Delgado *et al.*, 1990). The type of vegetation, climatic conditions and the species of grasshoppers may all be factors contributing to this difference. Based on these results further research should address the following:

1. formulation and application protocols
2. mode of infection
3. specificity of *B. bassiana* to certain species of grasshopper.

Nosema locustae

The spores of *N. locustae* used in the trials carried out in Cape Verde were produced by Evans-Biocontrol in the USA. After bioassay in the laboratory in Cape Verde to demonstrate viability of the spores, trials were set up in the field on Santiago island in September 1989, involving two doses of *N. locustae*: 2.5×10^9 spores/ha and 5×10^9 spores/ha. For the lower dose, six plots of 500 ha each were established in areas infested by grasshoppers, three treated and three controls. The dose of 5×10^9 spores/ha was applied to two plots, one of 200 ha and another of 100 ha with appropriate controls. Spores of *N. locustae* were applied in a bran-bait formulation with aerial application equipment provided by GTZ. In 1989, the assessment of results was based on evaluation of population density, using the ring method (0.1 m²) and disease prevalence. The assessment of

population density was begun two to three days before application and continued for 41 days. Disease prevalence was based on percentage of grasshoppers infected in collections of 100–150 grasshoppers each week near the centre of each plot. These samples also were used to determine the species composition of the grasshopper populations, the age structure of the grasshoppers and infection by other micro-organisms. Only *O. senegalensis* and *D. axillaris*, which were the most common grasshoppers, were examined for *N. locustae* and other micro-organisms. The observations were continued during the rainy season in 1990 (August–November).

Results and conclusions

The evaluation of population composition and density did not show any significant differences between treatments and controls. However, analysis of data on population composition and density of *O. senegalensis* showed a significant difference ($P<0.05$) on the 27th day in 1989. Also, in the 100 ha plot in the north of the island that was treated with 5×10^9 spores/ha we noted a distinct reduction in population in the treated plot compared with the control. However, the difference was not significant.

Since *O. senegalensis* was the predominant species in plots treated with 2.5×10^9 spores/ha, representing about 90% of all grasshoppers, observations in 1989 for prevalence of *N. locustae* were restricted to this species. Approximately 10,000 grasshoppers were examined. The first cases of infection by *N. locustae* were noted 27 days after treatment. As indicated in Fig. 5, the maximum prevalence of infection was about 18%. Infections were not observed in grasshoppers from control plots which demonstrates that infections resulted from treatments.

For the plots treated at 5×10^9 spores/ha, observations were made on *O. senegalensis* and *D. axillaris*. The level of infection in *O. senegalensis* by *N. locustae* was, on average, over 28% (Fig. 6). Infections were not observed in *D. axillaris*.

Observations were made one year after treatment (1990), both in plots treated with 2.5×10^9 spores/ha and in control plots, both of which showed general decrease in grasshopper population density from the previous year. There were no significant differences between treated and untreated plots.

The level of infection of *N. locustae* one year after treatment was lower than that in 1989 when the trial was begun.

The results obtained show that the dosages of 2.5×10^9 and 5×10^9 spores/ha of *N. locustae* did not significantly reduce population density of *O. senegalensis*. One of the reasons for this may be the very rapid development of *O. senegalensis* compared with the time necessary for *N. locustae* to reach a level sufficient to kill the insect. These results have however confirmed that *O. senegalensis* is sensitive to *N. locustae*, as previously

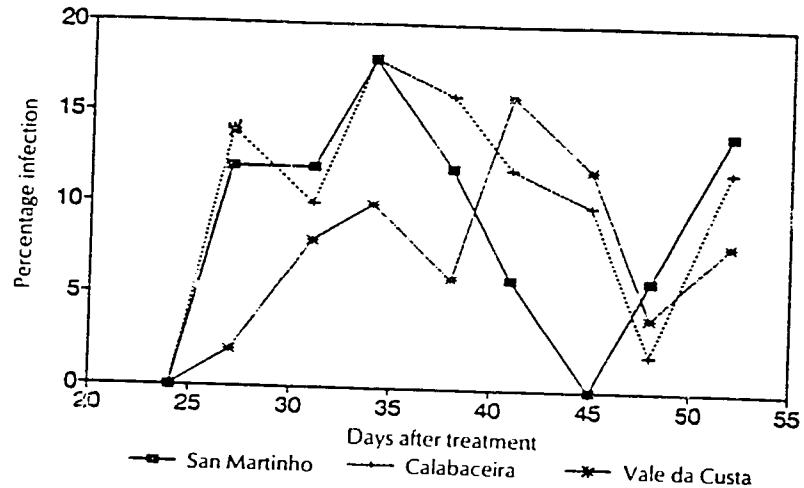

Figure 5. Infection levels of *Nosema locustae* in *Oedaleus senegalensis* (dose 2.5×10^9 spores/ha)

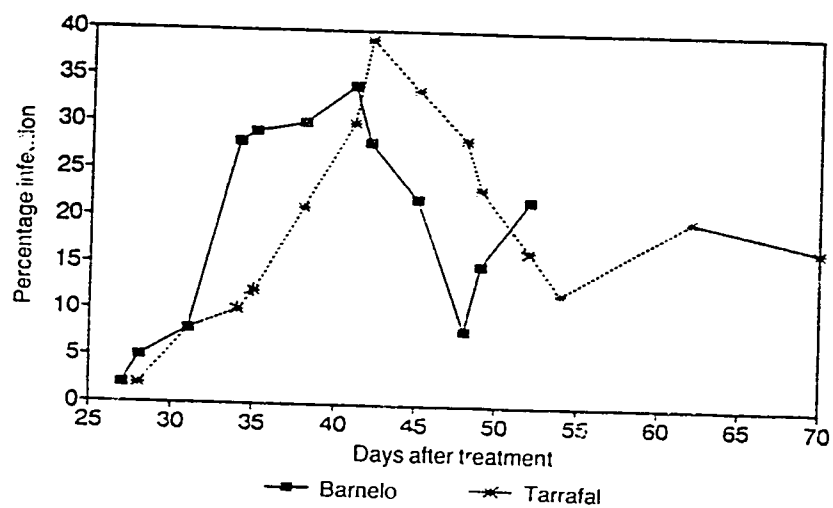

Figure 6. Infection levels of *Nosema locustae* in *Oedaleus senegalensis* (dose 5×10^9 spores/ha)

described by Henry *et al.* (1985a). The absence of infection in *D. axillaris*, which is also sensitive according to Henry (1991) and Henry *et al.* (1985a), was probably due to the unacceptability of the baits to this species.

In conclusion, large scale applications of *N. locustae* resulted in infections in *O. senegalensis* which possibly reduced densities, but the reductions are not sufficient for control of *O. senegalensis* in Cape Verde.

Viruses: evaluation of infection of *O. senegalensis* and *D. axillaris* with entomopoxviruses

Towards the end of 1990 two shipments containing cadavers of grasshoppers infected with OPV or MPV viruses were received from Montana State University (USA).

In the laboratory, extractions of these micro-organisms from the cadavers provided inoculum for bioassays in Cape Verdian grasshoppers and for mass production of these viruses to produce inoculum for subsequent field and laboratory tests.

Bioassays

Third instar nymphs were separated, placed individually in small glass tubes and starved for 24 hours. The inoculum in an aqueous suspension was placed on 7 mm discs of maize (or bean) leaves, using a 10 ml syringe. When the drops were dry, the leaf discs were fed to the grasshoppers. Only those grasshoppers which completely consumed the leaf disc were used in bioassays.

The following dosages were used in the bioassays: 10^6, 10^5, and 10^4 spores/insect (three replicates). After inoculation, the grasshoppers were placed in tubes in groups of five nymphs. The dead grasshoppers were removed daily from the tubes, titrated and homogenized in 5 ml of distilled water and the homogenate examined under the microscope for virus inclusions.

After 21 days, the remaining live grasshoppers were sacrificed and each was examined as above. The prevalence of infection noted at 10^6 varied between 16% and 35%, which established that *O. senegalensis* is susceptible to OPV infections. However, the high level of mortality provoked by other factors considerably reduced the number of grasshoppers at the beginning of the experiment, in treatments as well as in controls. Accordingly, another trial in the laboratory using other methodology is needed to obtain more conclusive results.

An identical study was tried with MPV against *D. axillaris*. However, the fact that infections were not evident, suggested the MPV does not infect this species.

In addition to the bioassays, grasshoppers were inoculated in order to mass produce these viruses. These inoculations resulted in production of a considerable quantity of OPV in *O. senegalensis*. However, again no infections occurred among the *D. axillaris* inoculated with MPV.

Isolation and evaluation of endemic organisms in grasshoppers in Cape Verde

A considerable number of grasshoppers (over 7,000 specimens) were collected on the islands of Santiago, Maio, Boavista and Santo Antao for disease diagnosis. Infection was observed in *O. senegalensis*, *D. axillaris* and other species by eugregarines (Protozoa) as previously observed by Henry *et al.* (1985b). These organisms exert little influence on grasshopper mortality. Some grasshoppers, especially *O. senegalensis* and *D. axillaris*, were parasitized by the protozoan *Malamoeba locustae* (King & Taylor). This protozoan causes debilitative effects that reduce the reproductive capacity of grasshoppers. A small number of *O. senegalensis* collected from different locations were infected with various fungi, which in one case was tentatively determined as *Metarhizium anisopliae* (Metschnikoff) Sorokin. Also, a fungus has been isolated from *D. axillaris* from Santo Antao island which is being studied for species determination. A microsporidian has been isolated from *Pyrgomorpha cognata* Krauss from Santo Antao and Santiago islands which is possibly an undescribed species. Also, endemic infections by an entomopoxvirus, which probably is the same as OPV that is being tested, has been observed in *O. senegalensis* from Santiago island. In addition, infections by the bacterium *Bacillus* (probably *B. cereus* Frankland & Frankland) have been diagnosed in grasshoppers from Santiago island.

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Field trials with the entomopathogenic fungus *Beauveria bassiana* against grasshoppers in Mali, West Africa, July, 1990

D.L. Johnson[1], M.S. Goettel[1], C. Bradley[2], H. van der Paauw[3] & B. Maiga[4]

[1]*Agriculture Canada Research Station, Lethbridge, Alberta:* [2]*Mycotech Corporation:* [3]*Ciba-Geigy AG:* [4]*Service Nationale de la Protection des Végétaux Ministère de l'Agriculture, Mali.*

Abstract

Spores were applied to open field sites and field cages near Mourdiah, Mali. Hand-held spinning disk nozzles (Micro-Ulva) were used to spray spores in dormant oil. For the field trials, grasshoppers were sprayed in replicated plots in 5 randomized complete blocks. Fifteen plots of 0.5 ha each were established, sampled for grasshopper age and density, and treated with spores and oil, oil alone or no treatment. Excellent spore viability (averaging 88%), grasshopper densities of 30 or more immatures per m^2, and optimum application conditions favoured a useful test. Grasshoppers were successfully infected in the field plots, although post treatment hatching and rainfall tended to obscure detection of infection and impact on population density.

Incubation of dead hoppers at high humidity confirmed the presence of *B. bassiana* in insects dying 4–14 days after treatment. Two weeks after field treatment, total mortality in cages of grasshoppers from plots sprayed was 72%. Hoppers collected from control plots had a total mortality of 22% when held in cages over the same period.

Cage trials included sprayed and unsprayed hoppers, collected and treated at the site, that were monitored in 60 field cages and assayed for infection. The results of these cage trials indicated that, over the 19 days following treatment application, mortality of grasshoppers sprayed with spores was approximately double the natural mortality experienced by control groups. Spores sprayed directly onto vegetation before grasshoppers were added to the cages did not result in significant mortality on most sampling dates. Direct contact spray treatment, resulted in significant mortality in samples of grasshoppers caged after spraying. However, no direct reduction in population was noted in this trial.

Introduction and background

Locusts and grasshoppers have caused considerable losses to African crops in both modern and traditional agricultural systems. Existing control strategies rely on chemical insecticides to prevent damage to vegetation (Table 1), and these methods, though often effective, are not always appropriate. Effective alternatives that offer improved safety could have rapid and favourable environmental and economic impact.

The use of pathogens may offer an environmentally sound method for the management of grasshoppers and locusts. Hyphomycete fungi are the most promising candidates (Prior & Greathead, 1989) because in addition to causing mortal diseases in many insects, they are usually cheap to produce and are available from commercial sources in quantities large enough for experimental purposes, and they are generally not pathogenic to non-target organisms (Goettel *et al.*, 1990). They can penetrate directly through the cuticle and do not necessarily need to be ingested in order to initiate disease. Recent research at Lethbridge and Butte indicates that grasshoppers are highly susceptible to some isolates of the fungus *Beauveria bassiana* (Balsamo) Vuillemin.

There are several records of *B. bassiana* infecting Orthoptera (MacLeod, 1954; Humber & Soper, 1986; Li, 1987; Moore & Erlandson, 1988). Some recent experimental studies have also demonstrated its pathogenicity to grasshoppers under laboratory conditions (Marcandier & Khachatourians, 1987; Moore & Erlandson, 1988). Other recent research has shown that grasshoppers are highly susceptible to a strain of *B. bassiana* isolated from a dead grasshopper in Montana (Johnson *et al.*, 1988). Grasshoppers that were fed wheat leaves treated with spores died within 10 days. In subsequent laboratory dose-mortality tests, spores of *B. bassiana* sprayed in water or applied on lettuce disks were found to infect and kill grasshoppers of other species and ages at economically reasonable levels of application. Experiments in which third-instar grasshoppers were

Table 1. Amounts and value of insecticide used for control of grasshoppers and locusts in Mali, 1986. (Rachadi (1986; cited by Kawasaki (1990)).

Product	Value (1,000 FFr)	Quantity
Diazinon 960	4,130	56,400 l
Fenitrothion ULV 500	11,379	199,625 l
Fenitrothion ULV 960/1000	2,637	35,400 l
γ-HCH 25 (BHC)	5,140	514 t
Malathion ULV 960	367	10,000 l

fed lettuce disks treated with droplets of spores indicated mortality of over 60% at one million spores per hopper. Reasonable field application rates (such as in the Mali field trial described below) would result in an average of over 1,000 times this quantity of spores per m^2. Economic analysis by Mycotech has indicated that, if successful, an application rate of 10^{13} conidia per ha can be competitive in price with chemicals currently used for grasshopper control.

The development of methods for the mass production of this strain by Mycotech Corporation has allowed preliminary field trials to be conducted against grasshoppers in Alberta, Montana, and Saskatchewan (Bradley, M.A. Erlandson, Goettel & Johnson, unpublished data). These applications included treatment of caged grass and crop, and also treatment of whole fields, using bran bait and spray formulations.

Objectives of Mali trials

Development of *B. bassiana* as a microbial insecticide effective for grasshopper control in Africa is a complex undertaking that will require an extended development and testing programme. The 1990 trials in Africa were a first step in this development programme and had several objectives:

1. to evaluate formulation and application methods under African conditions in field and cage trials
2. to test effectiveness against mixed species population of African grasshoppers
3. to gain experience in use of hand-held ULV sprayers for the purpose of fungal spore application.

Materials and methods

Conidia production and formulation

The *B. bassiana* isolate used in this study was originally obtained from a migratory grasshopper (*Melanoplus sanguinipes* (Fabricius)) collected near Three Forks, MT, USA, and is being developed by Mycotech Corporation, Butte, MT, for control of grasshoppers. Conidia were produced according to solid culture technology developed by Mycotech Corporation (Bradley *et al.*, in press; Goettel & Roberts, these proceedings). Conidia yields and concentrations were consistent, ranging from 1 to 3 × 10^{13} conidia per kg of dry substrate, and producing conidia powders ranging from 2 to 7 × 10^{10} conidia per g. Final blended conidia powders contained 4 × 10^{10} viable conidia per g. The conidia were transported to Africa as dry

powder in screw cap plastic bottles. The powder was held in refrigeration in Bamako and Mourdiah, Mali, but was otherwise held at ambient temperatures when taken to the field.

Conidia powders were formulated in oil on site immediately prior to field application. The oil (Sunspray, Sun Refining and Marketing Co., Philadelphia) is an inert paraffinic type and is registered with the EPA as an insecticide carrier. The oil formulation had been previously tested as a concentrate diluted in water, but to achieve ultra-low volume (ULV) application, it was necessary to use a suspension of undiluted oil and conidia. Greater infectivity of oil over water formulations was previously demonstrated by Prior *et al.*, 1988. The conidia were suspended in the oil at a rate of 100 g/1(w/v).

Conidia viability

Both the dry conidia powder and the formulated oil suspensions were assayed before and after application. To determine viability of the raw spore product, conidia powder was suspended and diluted in sterile water. Oil formulated conidia were diluted in oil. Aliquots of 0.1 ml containing approximately 1 × 10^4 conidia were spread onto the surface of Sabouraud's dextrose agar supplemented with 2% yeast extract, streptomycin (50 µl/ml) and penicillin (25 I.U./ml) in a 10 cm petri dish with a sterile glass rod and incubated at room temperature. After 7–10 h, the numbers of germinated and non-germinated spores were counted using a compound microscope. Conidia in oil were first stained by placing a drop of lacto-fuchsin (Carmichael, 1955) onto the surface of the agar and covering with a glass cover slip.

Ultra-low volume spray application tests

Preliminary application tests were conducted in Lethbridge to evaluate ULV application of the oil formulation. Micro-ulva sprayers manufactured by Micron Sprayers Ltd, Bromyard, England, were provided by Ciba Geigy AG, Basel. These sprayers are hand-held, and use a battery-powered spinning disc fed by gravity, from a 500 or 1,000 ml reservoir. Flow rate is determined by the orifice size of the nozzle that feeds the disc. Droplet size is determined by disc rotation speed, controlled by the number of batteries and by the viscosity of the formulation. Using the largest available nozzle size and a mid-range of 7,000 rpm (red nozzle, 4 batteries), the flow rates of oil containing various concentrations of conidia powder were determined. Droplet size and conidia dispersion were determined using oil sensitive spray cards and microscopic examination of droplets on glass slides. The cards and slides were treated by walking a 2 m swath in still air, using 70 g/l conidia suspension.

Mali field trial

Site description and experimental design

The field trial site was approximately 5 km from the village of Mourdiah, about 250 km north of Bamako. The terrain was flat and vegetation consisted of areas of trees and scrub intermixed with open areas. Open areas were a mix of grass cover and sandy areas without vegetation.

The trial was replicated in 5 complete blocks. Each block contained three 0.5 ha plots. The blocks occupied open areas, with the exception of the first block which was a mixture of trees with interspersed grass and open, sandy areas. Plots measured 70 m × 70 m and were laid out on compass bearings using a measuring wheel. The plots were separated from each other by a minimum barrier strip of 30 m. In each block, one plot was an untreated control, one plot was sprayed with formulation oil only, and the third plot received a spray application of spores in oil.

Field application

Application volume was 1.5 l/0.5 ha plot, giving an application rate of 1.2 × 10^{13} conidia/ha. Application rates were verified by collecting the formulated oil from the sprayer and counting spore density by using a haemocytometer. Oil only control plots were treated at the same spray volume. All plots were sprayed using Micro-ulva sprayers, with 4 batteries, with 1-3 sprayers in use simultaneously. Swath width was approximately 5 m. Wind speed was measured at the time of application, and plots were sprayed only if wind speed was less than about 2 m/s. All plots were sprayed in the evening between 17.00 and 19.00 h, with the exception of plot 3B which was sprayed beginning at 10.00 h. Control and test plots were sprayed on successive evenings on 14–17 July. Because of rainfall after treatment application (30 mm and 6 mm received on 14 and 16 July, respectively), plots were resprayed on 21 and 22 July. (In the results, these are referred to as the first and second spray applications.) An additional 38 mm of rain was recorded 23 July. Air temperatures during the trial ranged between 23 and 33°C.

Grasshopper population monitoring and mortality assessment

Grasshopper population species composition was determined from sweepnet samples taken prior to spraying. Grasshopper densities were determined using sample rings of 0.1 m^2 each, with 25 rings arranged diagonally across each plot. Population estimates were recorded by approaching and counting grasshoppers in each of the 25 rings, and an additional confirmative measure was made by counting the number observed in 25 visual m^2 quadrats. Sample rings were counted prior to spraying and at three-day intervals after spraying. Because of problems with passing livestock herds, it was not possible to keep track of the identity and position of individual ring counts. The pre-treatment means for each of the 15 plots were calculated, and this value was subtracted from each individual post-treatment observation to adjust the data to absolute changes from the original mean population density for the respective plots. The result was a set of DELTAs, where

for date i, block j, plot k, ring l, the deviation is

$$\text{DELTA}(ijkl) = \text{COUNT}(ijkl) - \text{COUNT}(0jk.),$$

that is, the lth count minus the initial kth plot mean. COUNT is the mean number of grasshoppers counted per 0.25 m^2 ring, and DELTA is the mean deviation from the pre-treatment ring count mean (date 0). A positive DELTA indicates a population increase; a negative DELTA indicates a decline relative to the initial grasshopper density. The DELTAs were subjected to analysis of variance, with F-tests against appropriate experimental error and sampling error mean squares.

Plots were searched for samples of dead grasshoppers beginning three days after application. *B. bassiana* infection was confirmed by incubating cadavers collected from the plots in petri dishes with moist cotton and observing for conidiation of the fungus. In addition, grasshopper samples were collected from test and control plots 0-3 days after spraying (using sweepnets) and held for daily observation in screened cages.

Vegetation and soil samples were collected from the site before treatment, and screened for *B. bassiana*, using a selective growth medium (Dodine Oatmeal Agar) (Beilharz *et al.*, 1982)

Cage trials

Montana

A preliminary cage trial was conducted by Mycotech Corporation near Three Forks, Montana, in late June, 1990. The objectives were to test the undiluted conidia preparations under field conditions and to test conidia preparations and formulation oil to be used in Mali trials.

Two application rates and two methods were tested. Application rates were equivalent to 1 × 10^{13} and 2 × 10^{13} conidia in 3 ha, applied to plots of 20 m^2 each. To assess direct contact, plots containing grasshoppers were sprayed. Grasshoppers were removed from these plots and placed in cages on unsprayed vegetation. Secondary contact was tested by placing unsprayed grasshoppers in cages on previously sprayed vegetation. There were replicate cages for each conidia application rate and application method.

Cages were observed daily by Mycotech personnel for seven days. At seven days after application the remaining living grasshoppers were removed to laboratory containment for continued observation and confirmation of *B. bassiana* infection.

Mali

Cage trials were conducted to evaluate direct contact versus contact with sprayed vegetation. The conidial suspension was made to be approximately equivalent to suspensions used in field trials. The suspension contained 2.3 $\times 10^9$ conidia/ml, determined by microscopic count. Grasshoppers were field-collected and were of mixed species composition and age. Cages covered 1.0 m^2 of ground surface and were 0.5 m high. They were constructed at the site from cloth netting over wooden frames. The netting could be opened at the top for introducing grasshoppers and for purposes of observation. Stakes and a soil berm around the bases held the cages in place and prevented grasshoppers from escaping under the netting.

The cage trials each included 4 treatments and two controls as shown below:

1. Cage 1 Control – no spray
2. Cage 2 Control – grasshoppers sprayed with oil only
3. Cage 3 Grasshoppers sprayed, vegetation unsprayed
4. Cage 4 Grasshoppers sprayed, vegetation sprayed
5. Cage 5 Grasshoppers unsprayed, vegetation sprayed
6. Cage 6 Grasshoppers unsprayed, vegetation sprayed, 4x dose.

Each set of six cages was replicated 5 times, resulting in a total of 30 cages. Each cage held 25 grasshoppers. For contact spray, treatment 3, grasshoppers were released on a large rectangular sheet of plastic and sprayed in one pass with a Micro-ulva sprayer. Grasshoppers were recaptured and placed in cages on unsprayed vegetation.

For the 'vegetation spray' treatment, one pass with the Micro-ulva sprayer was made over the wooden frames of the cages before the netting was put in place. Unsprayed grasshoppers were then placed in the cages on the sprayed vegetation. Treatment 4 consisted of sprayed grasshoppers confined with sprayed vegetation. In treatment 6, a four-fold higher dose was sprayed on vegetation, by two passes over the cages, using a double concentration of conidia suspension containing 4.9 $\times 10^9$ conidia per ml.

The cage trial was set up and sprayed on 18 July between 18.00 and 19.30 h. High wind with light rain (16.5 mm) occurred at about midnight. Cages withstood the wind but in the morning were found to contain water. The experiment was continued as planned, but to confirm the results the entire cage trial was repeated with a new set of hoppers and treatments on 19 July (referred to in the results as Trial 2).

Results

Conidia viability and application rates

Conidia in powder were determined to be 90% viable at packaging in Butte, Montana. Viability of oil formulated conidia in Mali at time of field application ranged from 85 to 95% with a weighted average of 88%. There was no apparent effect on viability of conidia held in oil during the spraying operation (approximately 1–2 h).

Flow rates decreased with increasing spore concentration (Table 2). At 170 g/l significant plugging of the valve occurred. Consequently, the concentration adopted for field trials was 100 g/l requiring application of 3 l/ha to obtain an application rate of 10^{13} viable spores per ha. At this concentration, most droplets varied from 30 to 300 microns diameter, and conidia were dispersed in droplets with little evidence of clumping.

Examination of spore droplets on microscope slides placed in the sprayer swath indicated a typical density of 20–40 spores per oil droplet.

Mali field trials

At the time of application of the spores in oil, the dominant grasshopper species in the site east of Mourdiah were *Kraussella amabile* (Krauss), *Cataloipus cymbiferus* (Krauss) and *Hieroglyphus daganensis* (Krauss), more than 95% as first to third instars. Less common species, also primarily represented by first to third instars, included *Acorypha glaucopsis* (Walker), *Acrida bicolor* (Thunberg), *Kraussaria angulifera* (Krauss), *Oedaleus senegalensis* (Krauss), *Diabolocatantops axillaris* (Thunberg), and *Zacompsa festa* (Karsch).

Population density just prior to treatment application was 4.2 immature grasshoppers/0.25 m^2 (17 July, n = 150). Although some post-treatment hatching obscured detection of levels of infection, and ants were seen to remove dead and dying hoppers, some dead hoppers that produced *Beauveria bassiana* hyphae were found at the site. High variation, eclosion,

Table 2. Flow rates of conidia oil suspensions in Micro-ulva sprayers.

Conidial Powder in Oil (g/l)	Flow Rate (ml/min)
0 (oil only)	33
33	32
67	24
170	intermittent (plugging)

and only moderate impact of the treatment resulted in no significant discernable decline in abundance following spray application. Three days after the second application the increase in mean grasshopper density (DELTA) was higher in the untreated plots than in the plots sprayed with either spores (lowest increase) or oil only (Table 3, Tukey's HSD, $P < 0.05$), indicating a somewhat greater population increase in the untreated plots. Only Block 5 had lower DELTA in the control plot. After this date even this limited impact in the field plots was not evident. The effects of spraying oil only vs. spraying spores in oil did not differ significantly ($P > 0.05$). Evidence of recent eclosion and large numbers of newly-hatched hoppers (mostly *C. cymbiferus*, *H. daganensis*, and *K. angulifera*) were observed within days after both the first and second spray dates. After this first post-treatment sampling date, the differences among treatments were not significant.

Field-collected hoppers from *B. bassiana*-treated plots held in cages confirmed infection by *B. bassiana*. Incubation of dead hoppers at high humidity resulted in fungal growth and sporulation in cadavers dying between 4 and 14 days after treatment. Two weeks after field treatment,

Table 3. Mean grasshopper count in each plot 3 days after the second application. COUNT is the mean number of grasshoppers counted per 0.25 m^2 ring. DELTA is the mean deviation from the ring count mean at the beginning of the experiment. A positive DELTA indicates a population increase.

			COUNT		DELTA	
Block	Treatment	n	Mean	S.D.	Mean	S.D.
1	untreated	25	9.84	3.44	5.08	3.44
1	oil only	25	7.16	3.21	−0.72	3.21
1	spore spray	25	8.20	3.55	4.40	3.55
2	untreated	25	6.20	3.52	3.00	3.52
2	oil only	25	11.12	4.24	5.56	4.24
2	spore spray	25	7.40	3.45	1.64	3.45
3	untreated	25	12.96	5.57	8.12	5.57
3	oil only	25	13.84	6.55	6.20	6.55
3	spore spray	25	11.00	3.54	3.88	3.54
4	untreated	25	11.32	5.71	1.36	5.71
4	oil only	25	4.72	4.37	0.84	4.37
4	spore spray	25	11.24	3.97	0.84	3.97
5	untreated	25	9.32	3.48	7.12	3.48
5	oil only	25	9.92	4.07	6.40	4.07
5	spore spray	25	10.08	3.96	7.28	3.96

total grasshopper mortality in all treated blocks was 72%. Hoppers collected from control plots had a total mortality of 22% when held in cages over the same period.

Vegetation and soil samples were collected from the site before treatment, and screened for *Beauveria* spp., using selective growth media. Colonies subsequently examined turned out to be other taxa. No native *Beauveria* spp. were found at the site.

Cage trials

Montana

All treatments in the trials conducted in Montana by Mycotech Corporation showed significant infection and 80 to 100% mortality in 7 to 14 days. Infection occurred equally from both direct contact spray and contact with sprayed vegetation. The Mycotech trial confirmed the compatibility of the conidia preparation with the oil formulation to be used in Africa.

Mali

Sprayed grasshoppers confined in the cages suffered significantly higher mortality than the control groups (Table 4). The average percentage of the grasshoppers that remained over 19 days following treatment application is plotted in Figs 1 and 2 (Trials 1 and 2). Even though the rains may have interfered with Trial 1, the impact of the direct spraying of spores is evident in both trials. In both trials, spores sprayed onto hoppers provided significant mortality ($P < 0.05$) one week after application.

The pattern, but not the statistical significance, persisted in the later dates.

Discussion

The conidia powder was stable and did not lose viability over the course of the experiments; although there were no extended periods of extreme (greater than 35°C) temperatures during transport and storage, this indicates that the spores are not highly sensitive to environmental conditions while kept in dry form. Formulation in oil did not adversely affect viability. The oil provides a stable, liquid formulation in which conidia do not germinate. The hydrophobic conidia were also found to disperse well in the oil, assisting in evenness of application.

The oil provided a means of easy application via hand-held sprayers. Based on discussions with Ciba Geigy and plant protection staff in Mali

Table 4. Analysis of variance and comparison of mean percentages of hoppers remaining alive in the treatment cages. The F-tests indicate overall block and treatment differences (tested against experimental error, block*treatment interaction). Means with the same letter, within a particular trial and date, are not significantly different (Tukey's HSD, $P > 0.05$).

TRIAL – 1 DAYS – 7 (Block $P = 0.028$, Treatment $P = 0.002$)

Test	Treatment	n	% alive	S.D.
a	check	5	73.6	21.47
ab	oil on hoppers	5	58.0	12.33
b	spores on hoppers	5	36.8	21.61
ab	spores on veg 1x	1	60.0	—
ab	spores on veg 4x	5	48.0	15.23
b	spores on hop & veg	5	31.2	11.10

TRIAL – 1 DAYS – 9 (Block $P = 0.060$, Treatment $P = 0.009$)

Test	Treatment	n	% alive	S.D.
ab	check	5	41.6	2.19
a	oil on hoppers	5	56.4	13.45
ab	spores on hoppers	5	32.8	20.67
ab	spores on veg 1x	1	52.0	—
ab	spores on veg 4x	5	29.6	16.64
b	spores on hop & veg	5	24.0	8.00

TRIAL – 2 DAYS – 7 (Block $P = 0.249$, Treatment $P = 0.062$)

Test	Treatment	n	% alive	S.D.
a	check	5	68.8	20.86
ab	oil on hoppers	5	58.4	21.84
b	spores on hoppers	5	22.4	11.17
ab	spores on veg 1x	5	41.6	22.38
ab	spores on veg 4x	5	39.2	27.77
ab	spores on hop & veg	5	45.6	31.57

TRIAL – 2 DAYS – 9 (Block $P = 0.331$, Treatment $P = 0.002$)

Test	Treatment	n	% alive	S.D.
a	check	5	56.8	18.42
ab	oil on hoppers	5	45.6	8.76
c	spores on hoppers	5	15.2	9.12
abc	spores on veg 1x	5	30.4	13.15
bc	spores on veg 4x	5	25.6	15.13
abc	spores on hop & veg	5	38.4	17.80

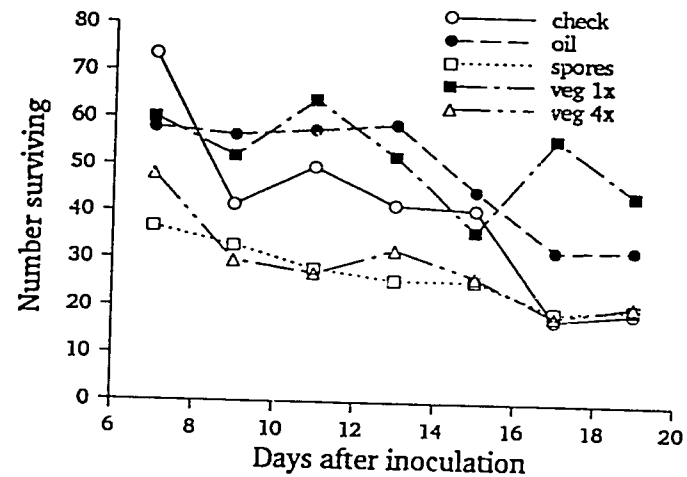

Figure 1. The average percentage of the grasshoppers that remained over 19 days following treatment application, Trial 1.

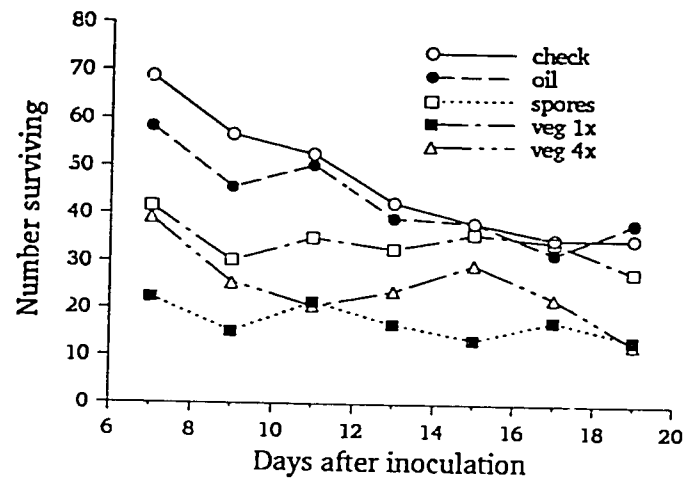

Figure 2. The average percentage of the grasshoppers that remained over 19 days following treatment application, Trial 2.

and Cape Verde, it was determined that ULV spinning disc sprayers would be the best application method for these initial trials. This type of sprayer is in widespread use in Africa, and they are also a model for ULV aerial application. The oil formulation was easily applied using this technology, although some difficulties were encountered due to variable winds and ground convection currents that occasionally hampered uniform application.

Pilot production data were evaluated by Mycotech Corporation in an economic model. The expected costs of solid culture technology production of spores to treat 1 million h at an application rate of 1×10^{13} conidia per ha are such that *B. bassiana* conidia could be profitably marketed. For example, the cost of 1×10^{13} conidia would be approximately US$5.00/ha, competitive with the midrange price of chemicals now used for grasshopper control.

Although no discernable decline in abundance of hoppers following field-spray application could be detected, indications were that infections were established and that there were greater population increases in the untreated plots. Post-treatment hatching, aggravated by movement of livestock herds through the experimental fields, obscured detection of any differences in population density between treatment and control plots beyond the initial sampling dates. On the other hand, 72% of the hoppers collected from treated plots 0–3 days after treatment died within 2 weeks of the treatment date, with confirmed *B. bassiana* occurring between 4 and 14 days. Although this may be an indication of actual mortality in the field, it must be taken into consideration that caged hoppers may be stressed and were possibly more susceptible to infection. In any case, these results demonstrate that the hoppers were at least exposed to the inoculum in sufficient quantity for infection to occur.

Results of cage trials confirmed that grasshoppers sprayed directly were susceptible to infection by the fungus. The results from the sprayed vegetation and vegetation plus hoppers are not as clear, but the direct spray treatment was significantly superior in most cases.

Results of subsequent trials in Cape Verde using the same conidia and oil preparations as in Mali (Delgado *et al.*, 1991) indicate that formulation attributes are very important. The Mali trials used only the oil formulations in field and the corresponding cage trials, whereas separate Cape Verde cage trials also included a wheat bran bait formulation. Sweep net samples from field plots taken 24 h after spraying showed a significant infection rate. In their trials, wheat bran bait in cage trials also showed significant mortality with greater than 90% kill in 14 days. However, field plot sweep net samples taken 72 h after spraying and cage trials of sprayed vegetation showed no significant mortality. The Cape Verde results agreed with our suggestion that grasshoppers did not become infected from contact with sprayed vegetation, but did become infected when contacted directly by spray and through consumption of spores on wheat bran bait. Preliminary cage trials in Montana showed that contact with sprayed vegetation was effective. A possible explanation for this discrepancy is that conidia may be less viable on vegetation surfaces in Africa than in Montana, for environmental reasons, such as intensity of sunlight. Another explanation is that grasshoppers may have avoided sprayed vegetation. This hypothesis is supported by preliminary laboratory bioassays in Lethbridge: 30% of *Melanoplus sanguinipes* starved for 24 h and presented with a lettuce disk treated with 5 μl of a conidia suspension in oil did not consume the lettuce whereas 100% accepted unsprayed control leaves (Bradley, Goettel & Johnson, unpublished data).

At this time, there is no explanation for the ineffectiveness of sprayed vegetation. Laboratory and environmental chamber studies will be necessary to determine differences between conditions in Montana and Africa. If further laboratory studies are pursued, they will be aimed at determining mode of pathogenesis and virulence as affected by formulation, inoculum targeting, host species and fungal strain.

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Field cage trials on the effects of enriched neem oil, insect growth regulators and the pathogens *Beauveria bassiana* and *Nosema locustae* on desert locusts in the Republic of Niger

O.M. Nasseh, T. Freres, J. Wilps, E. Kirkilionis & S. Krall

Deutsche Gesellschaft für Technische Zusammenarbeit GmbH.

Abstract

Field trials in large cages of 2 m² base area were carried out in the Tamesna area, northern Niger, in 1990–91, to test various spray and bait treatments against *Schistocerca gregaria* (Forskål). The insect growth regulator teflubenzuron was compared with a standard dieldrin treatment by direct application to *S. gregaria* adults and nymphs and *Anacridium wernerellum* (Karny) adults and also by application to the main food plant, *Schouwia thebaica* (Cruciferae) bushes. Pure and enriched neem oil sprays were tested for insecticidal and anti-feeding properties. Oil formulations of commercial preparations of *Beauveria bassiana* (Balsamo) Vuillemin were applied by spraying and *B. bassiana* and *Nosema locustae* Canning were also tested as baits with wheat bran. Teflubenzuron caused less adult mortality than dieldrin but completely prevented development of third and fourth instar nymphs. Neem oil caused no mortality by topical application but showed strong anti-feeding effects: however, severe burning of *S. thebaica* foliage by neem oil was also observed. None of the treatments involving spraying with *B. bassiana* or baiting with *B. bassiana* or *N. locustae* caused any mortality.

Introduction

Prior to the 1980s, organochlorines and other persistent pesticides were used extensively for locust control. Environmental and ecological problems were not seriously discussed until 1985 when swarms of *Schistocerca gregaria* (Forskål) reappeared in Africa and the Near East. Analysis of this recent outbreak showed that climatically favourable conditions in conjunction

with neglect of the warning services and the inaccessibility of some areas due to armed conflict had all contributed to the sudden increase. The countries affected were largely unprepared and poorly equipped to cope with the plague (Krall & Nasseh, 1991).

Since the use of pesticides is rigorously controlled in most temperate countries, but less so in many tropical ones, GTZ initiated a project to investigate and develop more acceptable alternative methods of controlling locusts and grasshoppers. In parallel with this, GTZ requested that trials should be initiated on the side effects of the new products on the environment and on non-target organisms. The first environmental study was performed in Cape Verde in 1989 and the second, on locusts, in Niger in September–October 1990 (Peveling & Weyrich, 1991 and these proceedings).

Field cage trials on alternative methods of locust control were carried out in Niger (Tamesna area) October 1990–January 1991 (Freres & Nasseh, 1991). Their purpose was to investigate the efficacy of the insect growth regulator teflubenzuron, neem oil and pathogens against desert locust.

Neem, *Azadirachta indica*, has been found to be a promising source of natural pesticides. Extracts possess distinct insecticidal and antifeedant effects against several species of insects including locusts (Wilps *et al.*; Langewald & Schmutterer, these proceedings). Since neem trees grow in Niger, it was decided to investigate the influence of enriched neem oil on adults of *S. gregaria*. The reasons for using neem are to minimize the contamination of the environment, to encourage the use of locally available plant material, to identify sources of botanical insecticides for commercial use and to replace or supplement existing synthetic insecticides against pests.

As early as the 1950s, *Beauveria bassiana* (Balsamo) Vuillemin has been field-tested on a small scale against a wide range of insects, and since the early 1970s it has been mass-produced in USSR and eastern Europe, mainly for control of Colorado potato beetle (*Leptinotarsa decemlineata* (Say) (Coleoptera: Chrysomelidae). The fungal biomass is usually ground and made into dusts or granules and in recent years separators have been used to collect pure conidia from dried cultures. Such products contain up to 1×10^{11} conidia/g and have usually been applied in water suspensions at high volumes. However, the possibility also exists for formulation into oil emulsion preparations for spraying at low or ultra-low volumes (Bateman; Bateman *et al.*; Johnston *et al.*; Matthews; Prior *et al.*; all these proceedings). The purpose of the oil would be to act as a 'carrier', ensuring sufficient spray volume to cover the target area adequately and to reduce evaporation of the droplets, which usually have a diameter of 70–90 μm. It has also been claimed that oils may reduce the rate of inactivation of the fungus.

Nosema locustae Canning, a microsporidian, has been developed as an insecticidal bait and tested extensively against grasshopper populations in the northern part of USA and also in Cape Verde. It is pathogenic to a wide range of acridids and infection has been shown to reduce feeding (Johnson & Pavlikova, 1986).

Methods

Site details

The trial area, at In-Sinkat, was 300 km north-west of Agadez and 40 km south of the GTZ base in Tamesna, northern Niger. This area is well-known as a recession area for *S. gregaria* during the dry season. The area is classified as between Sahelian and Saharan savanna with rainfall of 50–100 mm per annum (Freres & Nasseh, 1991). Vegetation occurs in discrete 'islands', surrounded by large tracts of sand. These islands provide shade, food, oviposition sites, protection from birds and also cover from extremes of temperature. The vegetation includes *Acacia* spp. (Leguminosae), *Panicum turgidum* (Gramineae), *Aerva javanica* (Amaranthaceae), *Tribulus* spp. (Zygophyllaceae) and *Citrullus colocynthis* (Cucurbitaceae). However, the dominant vegetation is the bush *Schouwia thebaica* (Cruciferae) which reaches a height of 1.5–2.5 m. A few of these vegetation islands harbour desert locusts during the recession time.

Adult *S. gregaria* collected from the field were counted, checked for abnormalities and kept under observation for one week. The population of *S. gregaria* in the collecting area was fairly stable (800–1000/ha). None were parasitized. The only other locust species seen in the trial plots was *Anacridium wernerellum* (Karny) which was much less numerous than *S. gregaria*.

Construction of cages

Experiments were carried out in 2 × 2 × 2 m cages with removeable sides. All the sides and the top were covered with removeable wire mesh (8 meshes/cm^2) which was fixed to the cross members. The cages were designed to avoid crowding, assist in counting and allow easy access (Nasseh & Freres, 1991). The lower 30 cm of the cage was made out of sheet metal and was sunk into the sand to a depth of 20 cm. In each cage there were two thinned *Schouwia thebaica* plants of uniform growth. The trials were replicated four times, using 30 adult desert locusts.* Assessments were made at daily intervals during the cooler morning time.

*No statistical analysis of the results was presented (eds).

Treatments

Insect growth regulators

Teflubenzuron ULV formulation was used at a rate equivalent to 50 g a.i/ha, a dose derived from previous trials in 1989 in Sudan where a dose of 25–50 g a.i./ha, was indicated. Where treatments were applied to the insects, the pesticide was applied outside the cages and the insects were transferred into the cages 30 min later. Where treatments were applied to the plants the insects were placed in the cages 30 min after spraying. Dieldrin ULV at 20 g a.i./ha was used as a standard insecticide and an untreated control was also included.

Enriched neem oil

This was obtained from Prof. H. Schmutterer, Justus-Liebig Universität Giessen, Germany. It was coded AZT-VR-K and originated from Togo and Tanzania. Pure neem oil from Niger was also tested. In the first trial, adult locusts were sprayed with enriched neem oil AZT-VR-K, enriched neem oil from Benin and pure neem oil from Niger at a rate equivalent to 10 l/ha. Thirty locusts were used in each treatment and the treatments were replicated four times.

In two other unreplicated trials on antifeeding effects, adult locusts were added to cages in which, in one trial, one *S. thebaica* plant had been sprayed with enriched neem oil at a rate equivalent to 10 l/ha 30 l min previously but the other had not, and in the other trial only one, sprayed plant was present. Assessments in all trials were made daily.

Pathogens

The *Beauveria bassiana* product (1×10^{13} conidia/kg) was obtained from Mycotech Inc. Butte, Montana, USA, (by courtesy of Dr C. Bradley) and was applied at two rates of 320 g and 960 g/ha in 3 litres mineral oil. *Nosema locustae* Canning (2.5×10^{9} spores on 1 kg of wheat bran/ha) was obtained from Durango Inc., Bayfield, Colorado, USA, and was compared with *B. bassiana* in a feeding trial where the latter was also applied on bran at rates for both pathogens equivalent to 320 and 960 g/ha. In these bait trials the locusts were starved for 24 or 48 h before treatment to enhance feeding. All these dosages refer to products, applied as received from the producers except the rate of 960 g/ha.

Application techology

A solar operated Micro-Ulva (Micron Sprayers Ltd., UK) was used for application in the early morning. Droplet distribution was assessed using oil sensitive paper.

Results

Insect growth regulators

Analysis of oil sensitive papers showed that the droplet deposition density was 20 ± 12 droplets/cm^2. The mortality caused by the treatments to *Schistocerca gregaria* (adults and third and fourth instars) and *Anacridium wernerellum* (adults) are shown in Tables 1–3.

The best control was obtained with dieldrin. Teflubenzuron gave better results when applied to the vegetation than to the insects.

The results obtained by applications of teflubenzuron to third and fourth instars of solitarious *S. gregaria* and by treatment of their food plant (*Schouwia thebaica*) are shown in Table 2.

Table 2 shows the number of surviving, dead and deformed (abnormal wings and tarsae) and the number of so called mini adults (inhibition of normal adult development) resulting from application of teflubenzuron at 50 g a.i./ha.

Mean body weight of male larvae treated with teflubenzuron was approximately half that of untreated larvae. None of the deformed larvae developed normally and none were able to complete development. Thus teflubenzuron appeared more effective in controlling the larval stage of desert locust than the adults under these conditions.

Table 1. Effect of teflubenzuron and dieldrin on adult *Schistocerca gregaria*.

Product	Application rate	No. of *S. gregaria* at application	No. of *S. gregaria* after 17 days
Teflubenzuron (topical)	50 g a.i./ha	120	78
Teflubenzuron (vegetation)	50 g a.i./ha	120	88
Dieldrin	20 g a.i./ha	120	19
Control		120	119

Table 2. Effect of teflubenzuron on third and fourth larval stages of *Schistocerca gregaria*.

Product	Application rate	Initial no. insects	No. alive after 12 days	No. of 3rd/4th larval stages after 12 days		
				No. dead	No. deformed	No. mini
Teflubenzuron (topical)	50 g a.i/ha	120	30	77	13	0
Teflubenzuron (vegetation)	50 g a.i/ha	120	11	84	21	15
Control		118	118	2	0	0

Table 3. Effects of teflubenzuron on adults of *Anacridium wernerellum*.

Product	Application rate	Initial no. of insects	No. of insects after 17 days
Teflubenzuron (topical)	50 g a.i/ha	51	21
Teflubenzuron (vegetation)	50 g a.i/ha	51	10
Control		51	50

The data in Table 3 suggest that the intake of teflubenzuron from treated *Schouwia thebaica* plants was more effective against the adults of *A. wernerellum*, than topical application. Treatment of the vegetation reduced the population from 51 to 10.

Enriched neem oil

The results shown in Table 4 demonstrate that there was no effect of pure and enriched neem oil on adults of *Schistocerca gregaria* up six weeks after treatment.

In the unreplicated anti-feeding trial, the locusts consumed 100% of the leaves of the *Schouwia thebaica* in the untreated control but on the

Table 4. Effect of neem oil on adult *Schistocerca gregaria*.

Product	Application rate	No. of *S. gregaria* after treatment	No. of *S. gregaria* after 6 weeks
Neem oil Giessen*	10 l/ha	120	116
Neem oil Benin*	10 l/ha	120	117
Neem oil Niger	10 l/ha	120	117
Control		120	118

*enriched neem oil

plants sprayed with neem oil at 10 l/ha there was no feeding until day 11, by which time 100 out of the 120 adults had starved to death. After four weeks there was severe phytotoxic burning of the leaves of *S. thebaica* treated with enriched neem at 10 l/ha.

Pathogens

The results for all treatments with *B. bassiana* and *N. locustae* are given in Tables 5–7. Analysis of oil sensitive paper distributed during the application of *B. bassiana* showed an average droplet coverage of 20 ± 12 droplets/cm^2.

Table 5 presents the results of direct spraying of the desert locust adults. Only the combination of *B. bassiana* and neem oil had any effect within six weeks and this was only slight.

Table 6 indicates that all the adult locusts which fed on *Schouwia thebaica* treated with 3 l mineral oil/ha or on the non-treated controls survived, but the *B. bassiana* treatments also had very little effect up to six weeks after treatment.

In other experiments also, the application of *B bassiana* in either neem or mineral oil at a rate of 320 g in 3 l/ha had no effect on adult *Schistocerca gregaria* up to six weeks after treatment when it was sprayed directly on *Schouwia thebaica* bushes.

Adult desert locusts were fed on wheat bran mixed with *B. bassiana* or *Nosema locustae*. The results are presented in Table 7 and indicate that neither treatment caused any mortality within the six week period of recording.

Table 5. Effect of *Beauveria bassiana* on *Schistocerca gregaria* adults.

Product	Application rate	No. of *S. gregaria* at application	No. of *S. gregaria* after 6 weeks
B. bassiana + mineral oil	320 g in 3 l/ha	120	117
B. bassiana + mineral oil	960 g in 3 l/ha	120	114
B. bassiana + Neem oil	320 g in 3 l/ha	120	98
Mineral oil	3 l/ha	120	119
Control		120	119

Table 6. Effect of *Beauveria bassiana* on adults of *Schistocerca gregaria* after feeding on treated plants.

Product	Application rate	No. of *S. gregaria* after application	No. of *S. gregaria* after 6 weeks
B. bassiana + mineral oil	320 g in 3 l/ha	120	115
B. bassiana + mineral oil	960 g in 3 l/ha	120	111
Mineral oil	3 l/ha	120	120
Control		120	120

Conclusions

The results of the teflubenzuron experiments demonstrated a significant effect on development and mortality became obvious on treated plants. A similar trend was observed in the mortality of sprayed *A. wernerellum*. However, mortality was higher in the dieldrin treatments. Treatment of larval stages of desert locusts with teflubenzuron in the field has promise. Larvae of *A. wernerellum* were not included in the experiments but further tests will be carried out.

Enriched neem oil caused no mortality but was effective as an anti-feedant. Neem is known to alter locust behaviour in several ways (Wilps *et al.*, these proceedings). For 10 days after treatment, no adult locusts fed on

Table 7. Effect of *Beauveria bassiana* and *Nosema locustae* on adults of *Schistocerca gregaria* after being fed with treated wheat bran (results of 2 experiments.

Product	Application rate	Starvation period (h)	No. of *S. gregaria* at treatment	No. of *S. gregaria* after 6 weeks
B. bassiana + wheat bran	320 g in 2 kg/ha	–	120	118
B. bassiana + wheat bran	960 g in 3 kg/ha	–	120	116
Control (*B. bassiana* treatment)			120	120
N. locustae + wheat bran	2 kg/ha	24	120	120
N. locustae + wheat bran	2 kg/ha	48	120	119
Control (*N. locustae* treatment)	2 kg/ha		120	118

neem-treated plants and the majority died in this period, although the survivors did begin to feed on the eleventh day. Neem oil therefore shows some promise as a treatment within programmes for the integrated management of desert locusts and could help to reduce dependency on chemical pesticides. It can also be produced locally in sahelian countries, which would help to reduce control costs. Other vegetable oils such as cottonseed have also been used with encouraging results as repellants against *Myzus persicae* (Sulzer) and *Brevicoryne brassicae* (Linnaeus) (Homoptera: Aphididae), *Spodoptera exigua* (Hübner) (Lepidoptera: Noctuidae) and also thrips pests (Butler & Henneberry, 1990). However, the severe phytotoxic effects of enriched neem on *Schouwia* indicate a need for further investigations on the feasibility of using neem in this way.

Six weeks after application of treatments to caged locusts in the field, nearly all locusts survived in the controls. Survival was not significantly less in all *B. bassiana* treatments, either applied in mineral oil to the plants or to the insects, nor was any effect demonstrated when locusts were fed wheat bran treated with *B. bassiana* or *N. locustae*.

The results show that *N. locustae* and *B. bassiana* were not effective biological control agents against *Schistocerca gregaria* under the conditions

of these trials. The question of whether or not these two pathogens could contribute to reducing the larval population densities of desert locusts could not be answered because larvae were not included in the experiments but tests will be continued with larvae. The viability of the *B. bassiana* conidia was not checked.

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Effects of neem oil, *Beauveria bassiana* and dieldrin on non-target tenebrionid beetles in the desert zone of the Republic of Niger

R. Peveling & J. Weyrich

Universität des Saarlandes.

Abstract

Field trials on the impact of locust control agents on non-target arthropods were carried out in Tamesna, southern Sahara, Republic of Niger, in September/October 1990. The study was conducted within the framework of the GTZ-Project 'Integrated Biological Control of Grasshoppers and Locusts'. The effects of neem oil (0.1% and 0.2% azadirachtin), *Beauveria bassiana* (Balsamo) Vuillemin and dieldrin on the tenebrionid beetle *Pimelia angulata tschadensis* Koch were studied in standardized *in situ* cage trials. Additionally, neem oil and *B. bassiana* were tested in small scale field trials in typical *Schistocerca gregaria* (Forskål) (Orthoptera: Acrididae) habitats (*Schouwia thebaica* vegetation islands). Dieldrin and neem (0.1%) caused high mortalities in the cage trials. Neem (0.2%) induced an increase, dieldrin a decrease in activity of the beetles. Neither in the cage trials nor within additional laboratory tests could *B. bassiana* infections be observed in *P. angulata tschadensis*. Only topically inoculated *Trachyderma hispida* (Forskål) (Tenebrionidae) were infected. The field applications did not appear to cause any side-effects on the tenebrionid fauna, i.e., neither alteration of activity pattern nor lethal effects. The final results of these investigations (effects on other non-target arthropods) will be presented in subsequent publications. As key components of arid ecosystems Tenebrionidae could be useful indicator organisms to study ecotoxicological risks of insecticides prior to large scale field applications.

Introduction

For decades locust control has relied almost exclusively on the application of chemical insecticides. However the increasing knowledge about their

potential hazardous side-effects (related to their non-specificity and persistence) has recently promoted the search for environmentally sound alternatives and adoption of the conceptual approach of integrated pest control, which incorporates biological and biotechnological methods, has received increasing attention from experts.

The application of alternative locust control agents can by no means be considered *a priori* to be environmentally sound. As with chemical pesticides, they must undergo a careful and thorough evaluation of potential side effects prior to large scale field application.

This ecotoxicological risk assessment involves both laboratory and field trials, the former to work out principal physico-chemical and toxicological (or pathogenic) properties of the respective control agent, the latter being indispensable to study its field behaviour, and thus preferably carried out at an early stage of risk assessment programmes.

Because most alternative insecticides are not selective, adverse effects on non-target organisms are to be expected. The assessment of the impact on non-target organisms is one of the prime objectives of ecotoxicology. An in-depth risk assessment can only be achieved if non-target-specific ecological processes are understood. This is a formidable task in tropical and subtropical ecosystems, where many species have not yet been taxonomically described. For ecological monitoring of (large scale) conventional applications, when a prompt assessment is required, it is common practice to confine ecotoxicological studies to higher taxonomic categories (mostly order or family). This simplification ignores the fact that there are significant differences in ecology, behaviour and susceptibility to pesticides even between closely related taxa. As a result, severe effects at lower taxonomic levels might be camouflaged. The search for sensitive *species* as indicators of undesirable side-effects (e.g. incorrect applications) in further routine field applications of the same insecticide in the same area should therefore receive more attention Grant, 1989; Everts, 1990.

In the first phase of environmental impact studies, investigations should focus on identifying non-target species either directly 'ecologically linked' to the target (competitors and natural enemies such as parasites or predators) or occupying key positions within the respective ecosystem (e.g. pollinators, reducers). However, it has to be emphasized that the importance of certain species may be underestimated or overlooked simply because the structure of the ecosystem, the underlying ecological processes and inter-specific relations are not sufficiently understood. Once identified, key species could undergo further standard laboratory testing (toxicity tests) in order to predict ecotoxicological risks of the application of a variety of agents in the same area. These indication methods should not be understood as substitutes for synecological approaches and will have to be worked out together with appropriate field scale assessments during field operations.

In the southern Saharan recession areas of the desert locust (*Schistocerca gregaria* (Forskål)), tenebrionid beetles are characteristic elements of the ground dwelling coenosis. Isolated plant and animal communities, low species density and diversity, and simple food webs are specific attributes of these ecosystems. Due to their high abundance and their omnivorous trophic function Tenebrionidae dominate the flow of organic matter. Moreover, they are direct food competitors of desert locusts and, as Popov (1980) demonstrated for the Senegalese grasshopper (*Oedaleus sengalensis* (Krauss)), potential predators of locust eggs due to their partly hypogaeic way of life. Therefore, in the first phase of the study, tenebrionids were chosen for studying non-target effects of alternative acridicides. The following agents were tested:

1. two formulations of neem oil (seed extract of *Azadirachta indica*)
2. *Beauveria bassiana* (Balsamo) Vuillemin, an entomopathogenic fungus
3. dieldrin as a reference.

The potential of neem oil for the control of *S. gregaria* has so far been tested only in the laboratory. Schmutterer & Freres (1990) demonstrated its larvicidal effects. Furthermore, laboratory reared gregarious hoppers developed the typical anatomical and ethological characteristics of solitary locusts. *B. bassiana* was field tested against *Nomadacris septemfasciata* (Serville) as early as the 1930s in South Africa (Schaefer, 1936). Prior & Greathead (1989) recommended additional investigations of the potential of *B. bassiana* and other entomopathogens as alternative acridicides. Dieldrin used to be the most commonly applied insecticide during locust control operations. The environmental hazards of its use are still controversial (Müller, 1988; Everts, 1990) despite an almost universal ban on its use. Further field investigations of its ecotoxicological effects should therefore be carried out.

Study sites

The studies were conducted in the Iguidi region about 15 km south-west of the CINA (Centre National de Lutte Antiacridienne) station 'In-Abangharhit' (south-east Tamesna, 18°03'N/6°02'E). The landscape can be described as more or less flat gravel and sand deserts, which are quite regularly separated by lines of dunes up to 20 m high. The vegetation consists of low-growing herbs, which are distributed in isolated patches between the dunes. Vegetation cover is dense along the dunes where patches of *Schouwia thebaica* (= *purpurea*)* (Cruciferae) up to 70 cm high

* Taxonomy in dispute (ed.)

(cover ≈ 80% can often be found; smaller, less dense patches are found between the dunes. *S. thebaica* is dominant and constitutes the major food of *Schistocerca gregaria* in this area. Hemming & Symmons (1969) studied the germination and growth of this annual cruciferous plant. Other species such as *Fagonia* sp. and *Tribulus ochroleucus* (Zygophyllacease), *Citrullus colocynthis* (Cucurbitaceae), *Aerva javanica* (Amaranthaceae), *Pergularia tomentosa* (Asclepiadaceae) and *Aristida* sp. (Gramineae) are loosely scattered within the *Schouwia* stands. Similarly, the macrofauna is characterized by a small number of species, most of them birds such as *Hirundo obsoleta buchanani* (pale crag martin), *Cursoprius cursor* (cream-coloured courser) and *Corvus ruficollis* (brown-necked raven) or reptiles such as *Acanthodactylus boscianus*.

As an adaptation to the dry and hot conditions, most arthropod species are active during the night or at dusk. Tenebrionid beetles make up the largest part of the zoomass. *Blaps bifurcata mirei* Gredelli, *Prionotheca coronata coronata* (Olivier) and *Pimelia subquadrata chudeaui* Koch occur frequently, but are less abundant than *Pimelia angulata tschadensis* Koch and *Trachyderma hispida* (Forskål). As with *S. gregaria*, they feed mainly on *Schouwia thebaica*.

The cage trials were conducted in a plains area of about 5 ha mainly covered with *S. thebaica*. For the field applications three dense *S. thebaica* fields of about 500 to 1500 m² were selected on the lee side of sand dunes. For logistical reasons, the trial sites were chosen near each other.

Materials and methods

Spraying operation

In the cage trials the oral and residual effects of the following agents were tested:

1. neem oil (0.075% ≈ 0.1% AZA (azadiractin) (Inst. f. Phytopathologie, Giessen/FRG))
2. neem oil (0.22% ≈ 0.2% AZA (IITA, Benin))
3. *Beauveria bassiana* (oil-formulated suspension (Mycotech, USA))
4. dieldrin (Shell-Agro).

Neem oil (0.1%) and *B. bassiana* were applied in the field trials. Spraying was conducted between 06.00 and 10.00 h (windspeed < 2 m/sec, temperature ≈ 25°C) with a Micro-Ulva-spinning disc sprayer (dieldrin: ULV, other agents LV application) (see Table 1).

Table 1. Application parameters.

Formulation	a.i.	Dosage rate	Flow rate	Nozzle	NB
Neem oil	0.1 % (AZA)	10.0 l/ha	60 ml/min	red	4
Neem oil	0.2 % (AZA)	10.0 l/ha	34 ml/min	red	4
B. bassiana	4 • 10⁹ sp/ml	1 • 10¹¹ sp/ha	61 ml/min (•)	red (•)	5
Dieldrin	18 %	0.4 l/ha	22 ml/min	yellow	4

NB – number of batteries/sprayer; sp – spores; (•) – a constant flow of the suspension could only be achieved when widening the nozzle diameter and shaking the sprayer continuously during application; AZA – azadiractin.

Cage trials

In the *Schouwia thebaica* field 20 suitable 2 m quadrats with at least 15% vegetation cover were selected (minimum buffer zone between the plots = 15 m). After treatment (four replicates per insecticide, randomized cage order) the cages (steel frame, galvanized iron netting, 2 × 2 × 2 m size; cage design see Krall & Nasseh, 1990) were dug into the ground 20–30 cm deep. All arthropods in the cages were removed. Subsequently, each cage was stocked with 30 *P. angulata tschadensis*. Over a period of 16 days the following parameters were recorded:

1. mortality (M) (twice daily at 07.00 and 18.00 h.)
2. relative activity (RA) (i.e. percentage of beetles captured in one pitfall trap per cage; the traps were covered with a lid during the day and opened for one hour starting at 18.30 h).

At the end of the trial all remaining beetles were collected to assess potential losses by cannibalism or escape.

The calculations of mortality and activity correspond to the sum (S) of dead (D) or alive (A) test beetles collected until the end of the trial. The cumulative mortality on day i is calculated as:

$$Mi(\%) = \frac{Di}{S} \times 100$$

(Di = number of dead animals collected up to day i).

The effects of the agents were calculated according to Schneider-Orelli (Krall & Nasseh, 1990). The relative activity on day i is calculated as:

$$RAi(\%) = \frac{Gi}{S - Di} \times 100$$

(Gi = number of animals collected on day i).

Prior to statistical treatment all data were arc-sine transformed. In particular the following statistical calculations were performed:

1. mortality at the end of the trial (day 16): single classification ANOVA and Newman-Keuls-multiple comparison among means
2. RA of all days: cross correlation; days 3–15: Friedman-ANOVA and Wilcoxon-Wilcox-multiple comparison among means.

Small-scale field trials

In each *Schouwia thebaica* field 4 permanent 1 m² plots were randomly established to survey potential mortalities of non-target organisms. To study effects on the activity abundance AA (= 'Aktivitätsdichte' *sensu* Heydemann (1953), 'availability' *sensu* Southwood (1978)) of ground-dwelling arthropods, four pitfall traps were placed in each test plot (conservation fluid: 5% formol) for a period of 15 days (3 days pre- and 11 days post-spray interval).

Mortality (M) was recorded daily between 07.30 and 08.30 h, 17.30 and 18.30 h in the permanent plots, and pitfall traps were emptied daily between 17.30 and 18.30 h.

At present only effects on the abundant tenebrionid species, *Pimelia angulata tschadensis*, *P. subquadrata chudeaui*, *Trachyderma hispida*, *Blaps bifurcata mirei*, and *Prionotheca coronata coronata*, can be given.

All data were transformed as : $A_T = 10 \times (\ln AA + 1)$ [A_T = AA transformed]. The mean catches of days 2 and 1 (pre-spray) were compared to the catches of days 2–4, 6–7 and 9–11 (post-spray) (Kruskal-Wallis). The effect of the agents is calculated as

$$E(\%) = \left[\frac{D_T}{D_C} - 1\right] * 100$$

D_T = mean difference between pre- and post-spray catches in the treated plot

D_C = mean difference between pre- and post-spray catches in the control plot

For the calculation of standard error see Everts *et al.* (1985).

Additional trials

To study the contact effects of *B. bassiana* on several tenebrionid beetles field tests and bioassays were conducted:

1. LV-application in an arena: 1 ml spore suspension/m² (as specified in Table 1) applied with a Micro-Ulva
2. topical application in the laboratory: approximately 1 µl suspension applied dorsally between thorax and abdomen by micro caps.

The test animals were analysed by the BBA Darmstadt–Germany for infection by *B. bassiana*.

Results

Cage trials

91.3% of the original stock of *P. angulata tschadensis* were recollected. The 8.7% beetles undetected must be attributed to cannibalism. One out of four replications in the control group was proved significantly to be an outlier (Outlier text of Dixon as described by Snedecor & Cochran (1980)) and was treated as missing (statistical analysis for unbalanced design).

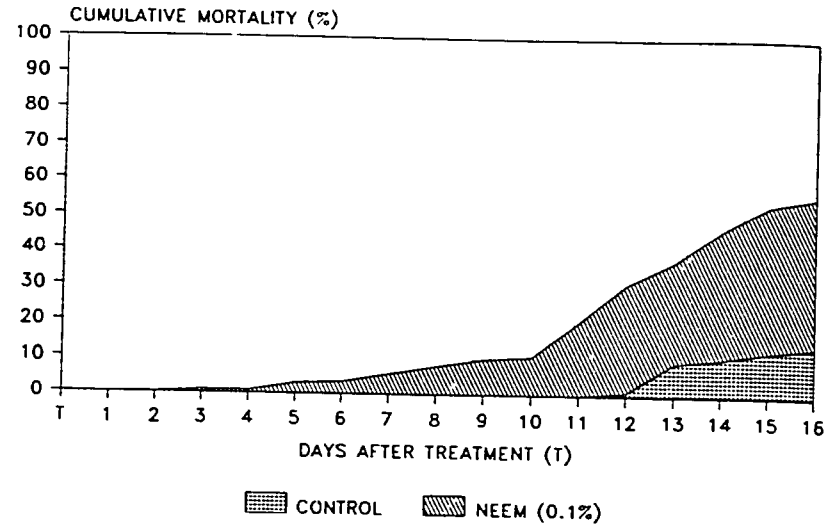
All test agents had an adverse effect (Table 2 and Figs 1 & 2), but only dieldrin ($P < 0.001$) and neem 0.1% ($P < 0.05$ caused significantly increased mortalities. Surprisingly, neem 0.1% finally caused higher mortalities than neem 0.2%, which is probably due to the different additives used in both formulations. No infection could be detected in the *B. bassiana*-treated beetles.

The activity of the beetles is cyclical (Fig. 3). Data from days 1 and 2 are slightly biased due to a prolonged sampling period (day 1: 90 min; day 2: 110 min). The periodicity of the data, although biologically important, is irrelevant for assessing the effects of the agents. This becomes evident when comparing the pattern of the activity of cage-tested tenbrionids in all replications (Table 3) and that of field-tested tenebrionids in all plots, respectively (see small-scale field trials, below). Differences in the effects of the agents are indicated by level of activity (Table 4) as compared to that of the control. Neem-treated beetles exhibit insecticide-caused hyperactivity whereas the dieldrin- and *B. bassiana*-treated groups exhibit hypoactivity.

Table 2. Mean mortality at the end of the trial.

Agent	Control	Neem (0.1%)	Neem (0.2%)	Dieldrin	*B. bassiana*
Mortality	14%	56%	48%	78%	37%
Corrected effect	–	49%	40%	74%	27%

Control ≠ dieldrin & neem (0.1%) ($P < 0.05$; ANOVA & Newman-Keuls multiple comparison among means); other differences not significant.



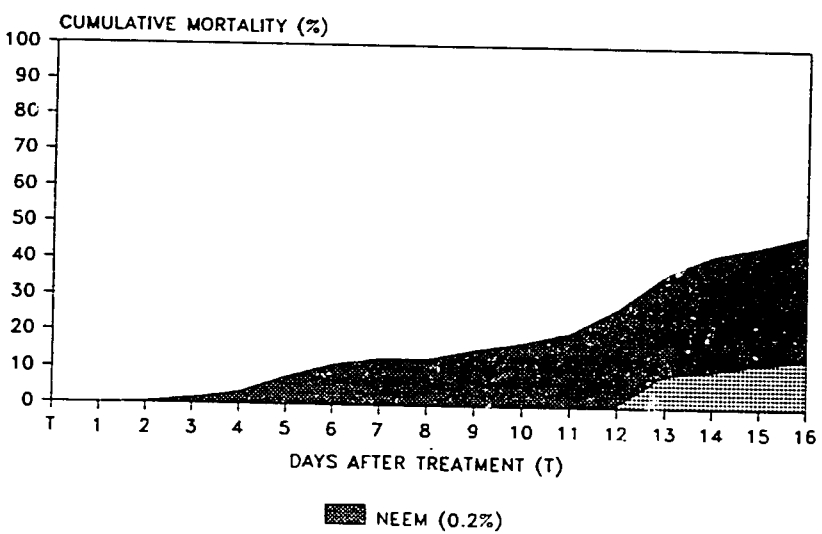
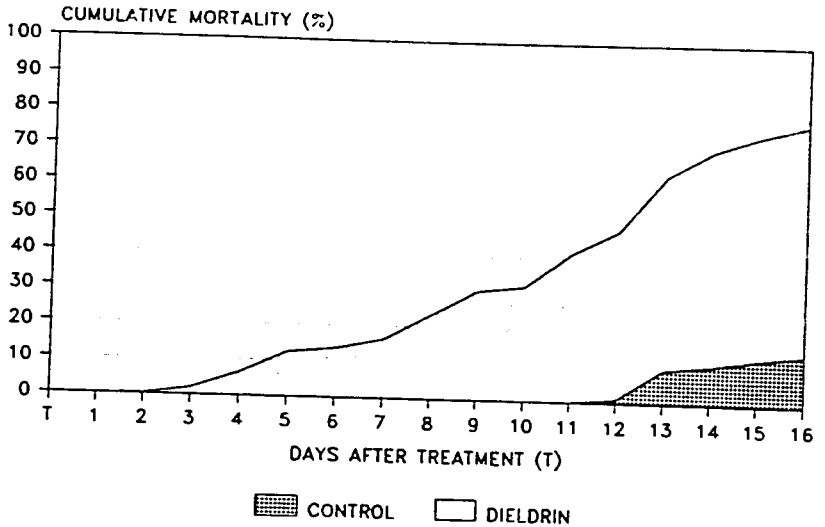


Figure 1. Cumulative mortality (M) of *Pimelia angulata tschadensis*; neem (0.1%) and neem (0.2%).



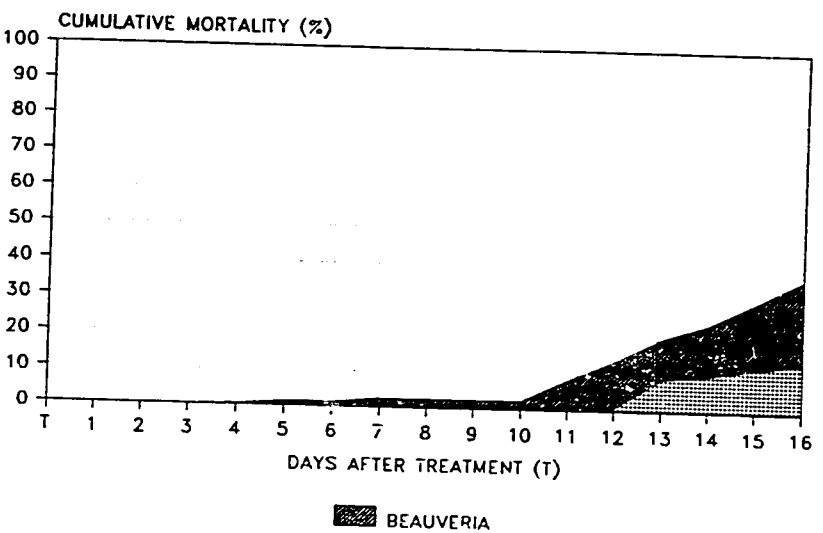


Figure 2. Cumulative mortality (M) of *Pimelia angulata tschadensis*; dieldrin and *Beauveria bassiana*.

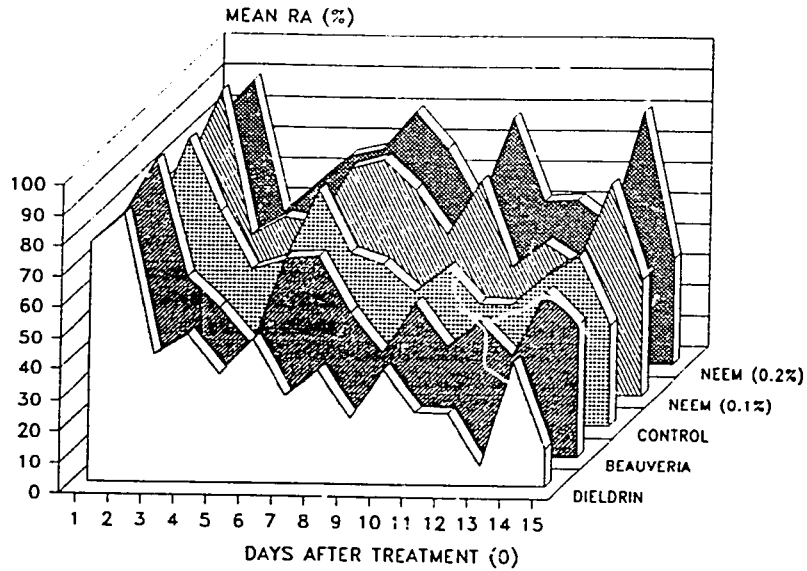


Figure 3. Mean relative activity (RA) of *Pimelia angulata tschadensis.*

Table 3. Correlation matrix (Pearson's r) of the mean relative activity (RA) (all days).

	Control	Neem (0.1%)	Neem (0.2%)	Dieldrin	*B. bassiana*
Control	1.00				
Neem (0.1%)	0.82***	1.00			
Neem (0.2%)	0.67**	0.95***	1.00		
Dieldrin	0.84***	0.82***	0.74**	1.00	
B. bassiana	0.87***	0.86***	0.75**	0.89***	1.00

Correlation coefficient significant at: ** $P < 0.01$; *** $P < 0.001$.

Small-scale field trials

The field trials did not indicate any adverse effects of neem oil (0.1%) and *Beauveria bassiana* on tenebrionid beetles. No dead animals, neither tenebrionids nor other arthropods, could be found on the permanent plots.

There was no difference in the activity of tenebrionid beetles (species group listed in small-scale field trials, above) between pre- and postspray periods (Table 5). As with the RA of the beetles in the cage trials, the

Table 4. Mean relative activity (RA) of *P. angulata t. schadensis* on days 3–15 (retransformed data).

Agent	Control	Neem (0.1%)	Neem (0.2%)	Dieldrin	*B. bassiana*
Activity	51%	56%	60%	32%	46%

Control ≠ Neem (0.1%), Neem (0.2%) & *Beauveria* ≠ Dieldrin; Neem (0.2%) ≠ *Beauveria* ($P < 0.05$; Friedman-ANOVA & Wilcoxon-Wilcox multiple comparison among means with days 3–15 as replicates); other differences not significant.

Table 5. Effect of the agents on the activity abundance (AA) in the field trials.

Agent	P	A(1)	E	SE	A(2)	E	SE	A(3)	E	SE
Control	62	49			41			38		
Neem (0.1%)	40	58	18.7	9.3	33	6.8	7.8	30	5.5	3.7
Beauveria	44	42	5.4	3.5	34	4.7	5.4	28	−0.6	3.2

Mean AA (number tenebrionids/trap) on days −2 & −1 (P), 2 to 4 (A(1)), 6 to 8 (A(2)) and 9 to 11 (A(3)); all differences not significant. (Kruskal-Wallis-ANOVA); E = effect, SE = standard error.

activity of the tenebrionids in the field tests is phased sequentially (Fig. 4; maximum on days -3, 1, 5, 8/9). This phenomenon can be shown clearly by a correlation matrix. A period of 5 days between the peaks is evident (Fig. 5 & Table 6).

The synchronous pattern of activity of the beetles is evident not only *within* the cage or the field trials, but also *between* these as illustrated in Fig. 6 and Table 7 (simultaneous testing period). Only during days 11–13 are the curves not synchronous (probably due to increased silting of the pitfall traps in the *Schouwia thebaica* fields).

The activity abundance of the tenebrionids in the control area (field tests) is higher on most days than in the treated plots (Table 8, Fig. 4).

Biotests

Both in the laboratory (constant 35°C test temperature) and in *in situ* topical application tests, *Pimelia angulata tschadensis* showed no *B. bassiana* infection (analysis by BBA, Darmstadt). Only in *Trachyderma hispida* could sporulation be detected (the beetles had died 4 to 8 days after treatment without showing any specific symptoms; also, under

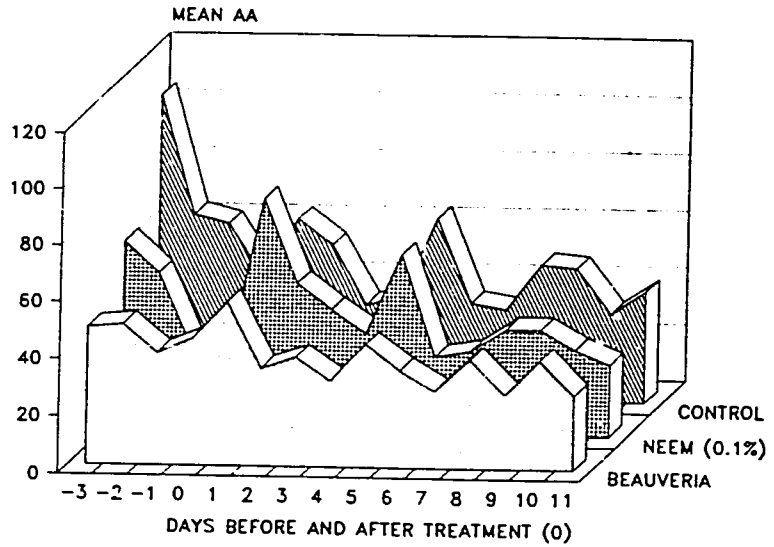


Figure 4. Mean activity abundance (AA) of tenebrionids (number of beetles/trap).

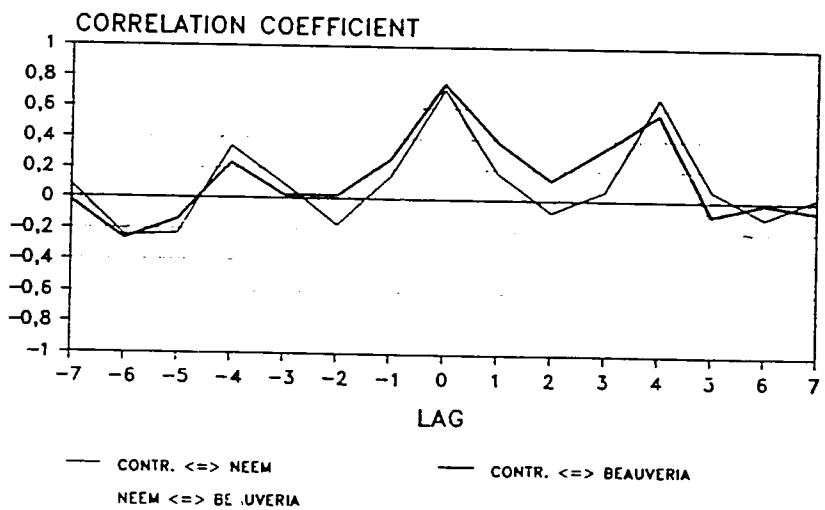


Figure 5. Correlation of the AA of tenebrionids in treated and untreated plots (LAG = 0, 1, 2, . . ., 7 means, that the AA in plot *a* on day *x* is correlated with the AA in plot *b* on day $x + 0, 1, 2, \ldots, 7$; for negative LAGs the same applies: $x - 0, 1, 2, \ldots, 7$); correlation coefficient > 0.6 or < −0.6 are significant at $P < 0.05$.

Table 6. Correlation matrix (Pearson's r) of activity abundance (AA) (all days).

	Control	Neem (0.1%)	*Beauveria*
Control	1.00		
Neem (0.1%)	0.73**	1.00	
Beauveria	0.76**	0.77***	1.00

Correlation coefficient significant at: ** $P < 0.01$; *** $P < 0.001$.

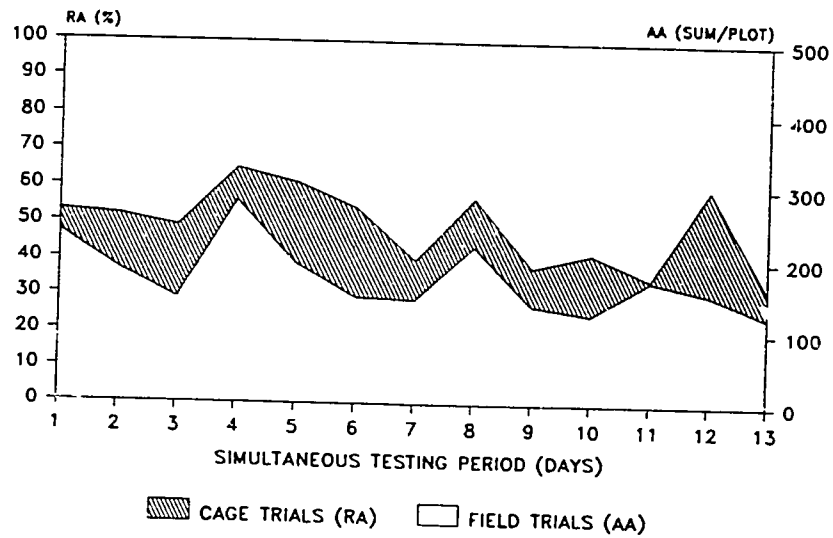


Figure 6. Mean AA in the field trials (total catch/day & plot) and mean RA in the cage trials (all replicated and treatments pooled) on simultaneous days.

Table 7. Matrix (Pearson's r) between relative activity (RA) (cage trials) and activity abundance (AA) (field trials) on simultaneous days.

AA	RA	Control	Neem (0.1%)	Neem (0.2%)	Dieldrin	*Beauveria*
Control		0.77**	0.52	0.38	0.63*	0.67*
Neem (0.1%)		0.86***	0.58*	0.34	0.46	0.75**
Beauveria		0.79**	0.35	0.15	0.53	0.54

Correlation coefficient significant at: * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

Table 8. Mean activity abundance (AA) of tenebrionid beetles (retransformed data).

Trial plot	Control	Neem (0.1%)	*B. bassiana*
AA	47%	39%	37%

Control ≠ Neem (0.1%) & *B. bassiana* ($P < 0.05$; Friedman-ANOVA & Wilcoxon & Wilcox multiple comparison among means, days 1–15 as replications); other differences not significant.

increased post-treatment humidity conditions the fungus did not break out within an 8-week observation period; only when beetles were kept in wet chambers did mycelia and spores develop).

It is remarkable that the oil-formulated spore suspension remained virulent for more than 10 days even under extremely arid conditions.

Discussion

The assessment of the impact of alternative locust control agents on non-target fauna requires a sound knowledge of the structural and functional components of the native ecosystem. Investigations of deleterious effects on parasitoids or predators of the target and on ecological key-species should be part of the risk assessment studies. With regard to the control of *Schistocerca gregaria* tenebrionid beetles can be defined as characteristic elements of the epigeal arthropod fauna of Saharan *S. gregaria* recession areas. It is not yet clear whether they are regulators of the density of desert locusts. However, they are both competitors for food and potential egg predators (Popov, 1980). Moreover, they represent a high percentage of the native zoomass. Their population reduction or, at worst, their extinction would probably cause severe disruptions of the mass and energy balances.

The cage trials indicated the ecologically hazardous potential of dieldrin. None of the other agents tested had comparable effects on *P. angulata tschadensis*. The significant effects of neem oil (0.1%) in the cage trials could not be verified in field tests. However, as with the cage trials, the activity level in the neem applied field test plot increased directly after treatment (see Table 5). This hyperactivity might indicate a repellent effect of neem (H. Schmutterer, pers. comm.). Over the whole observation period, the activity abundance in the control plot was higher than in the treated plots. This might be attributed to the different size of the *Schouwia thebaica* fields (control-plot ≈ 1500 m², neem-plot ≈ 1000 m² & *B. bassiana*-plot ≈ 500 m²), the larger ones having a higher carrying capacity. *Beauveria bassiana* did not have any effect on the tenebrionids. Even topical applications led only to infection of *T. hispida*. Tests on desert locusts which were carried out at the same time (Nasseh & Freres, 1991) gave just as little evidence of *B. bassiana* virulence. The low virulence may have been caused by the arid and hot conditions, by specific non-susceptibility, or by both factors together. The *B. bassiana* formulation tested does not seem to be fully developed. In addition, because of flocculation the suspension is not suitable for ULV applications. Periodic fluctuations of the activity are superimposed on potential treatment effects. Because of the periodicity (5-day intervals) and the consistent curve pattern of activity of the cage- and the field-tested beetles, these must be explained by external influences (for example, wind or humidity) and not by population dynamic factors in a narrow sense such as birth or death rates, gains from immigration, and losses from emigration. Insecticide-induced effects, even if statistically proved, should be interpreted with careful evaluation of ecosystem-specific characteristics. Consequently, a proper risk assessment can only be achieved if basic ecological research is included.

In recent ecotoxicological studies, due to a low susceptibility, Tenebrionidae did not appear to be good indicators of early effects of pesticide impacts (Müller, 1988; Van der Valk, 1990). Nevertheless, we believe that because of their high abundance and important ecological function in arid ecosystems as well as their potential for predating *Schistocerca gregaria*, the tenebrionids *Pimelia angulata tschadensis* and, to a lesser extent, *Trachyderma hispida* are appropriate test animals in early step laboratory investigations to assess the ecotoxicological risk of a wide range of chemicals in sahelian ecosystems.

Acknowledgements

We are grateful to Mrs F. Casci (Ministry of Agriculture, Fisheries and Food, London), Mrs B. Fliedner (Institut für Biogeographie, Univ. Saarbrücken, FRG) and Dr P. Matteson (Dept. of Entomology, Iowa State University) for reviewing the text.

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The effect of various neem products on the survival and flight activity of adult *Schistocerca gregaria*

H. Wilps[1], O. Nasseh[2] & S. Krall[2]

[1]*Forstzoologisches Institut, Universität Freiburg;* [2]*GTZ-Projekt Biologisch Integrierte Heuschreckenbekämpfung, Eschborn.*

Abstract

Schistocerca gregaria (Forskål) adults were collected in the Tamesna desert (Republic of Niger) and treated topically with various neem product formulations. Depending on the application method, untreated controls and locusts sprayed with palm oil did not show any mortality, but the treatment with neem oil usually decreased the flight performance by up to 70%. Recovery from this reduction could not be observed in flight experiments repeated at day 6 after application.

Injection of synthetic adipokinetic hormone did not raise the blood lipid concentration of neem-treated *S. gregaria* nearly as much as it did in untreated controls. Therefore, it can be inferred that compounds from neem seed kernels inhibit the lipid mobilizing system of *S. gregaria.* Consequently, the locusts treated with neem products have insufficient reserves to fly long distances.

Introduction

Energy metabolism of the locust is regulated by hormones stored in the corpora cardiaca (cc). The adipokinetic hormones (AKH) synthesized and released by the cc increase the concentration of energy-rich metabolites (lipids) in the haemolymph to provide flight muscle with energy required for long-distance flights. Release of the hormones is initiated at the onset of flight activity and the lipids originating from the fat body are completely oxidized by the flight muscle cells (Beenakkers *et al.*, 1984).

In various insect species neem products, as well as injected azadirachtin, interfered with the hormonal regulation of flight metabolism and reproduction (Schulz, 1985a, 1985b; Rembold *et al.*, 1986, 1988,

1989; Dorn *et al.*, 1988; Subrahmanyam *et al.*, 1989), and led to reduced or completely inhibited flight ability and fertility (Steffens & Schmutterer, 1982; Wilps, 1989; Schmutterer, 1990).

Most of the investigations on the effect of neem products as well as the mode of action of azadirachtin have been done under laboratory conditions. Additionally, there is no information on the effect of neem products on the adults of *Schistocerca gregaria* (Forskål).

In our experiments, adult *S. gregaria* collected and kept in field cages were used in tests carried out at In-Abangharit (Tamesna desert, Republic of Niger). The studies aimed to elucidate the fitness reduction of *S. gregaria* following the application of various neem products by different methods. The mortality rate and flight performance were chosen as the measured variables.

Materials and methods

The locusts were collected in Arabigou (Tamesna desert, Niger), an area of about 3.5 km^2 covered with *Schouwia thebaica* (= *purpurea*)* (Cruciferae), located 100 km to the north-west of In-Abangharit. The locusts were caught between October and November 1990 and reared in cages (2 x 2 x 2 m) set up in a field, also covered with *S. thebaica*, located near the base of In-Abangharit. When the locusts were caught, most of them were immature and showed an intermediate phase status. After three weeks, however, most had changed to the gregarious phase and also were fully developed. In the experiments only adult males were used.

Application on resting locusts (ground treatment)

Neem and palm oil were applied at a rate of 1 ml/m^2 (10 l/ha) by using an ultra-low volume (ULV) hand sprayer. To prevent *S. gregaria* from flying away they were exposed to cold (20 min at 6°C) before experimentation, and subsequently transferred into an area of 16 m^2 bordered with a plastic fence 1.5 m high.

Application during flight activity (flight treatment)

The locusts were fixed on a flight mill immediately before treatment with a dosage of 1 ml/m^2. The volume applied was calculated from the flow rate of the sprayer, the spraying time, and the ground area which was covered

*Taxonomy in dispute (eds).

by the spraying cloud. After application, flight activity continued for at least 3 min, at an average speed of 7.7 ± 0.4 km/h.

Measurement of flight activity

Six locusts were attached to a flight mill and, utilizing the fact that insects without ground contact show permanent flight activity, the number of revolutions was counted by a mechanical counter to obtain an average for all six. All experiments were carried out at temperatures between 28°C and 32°C. Flight was measured for periods of 30 or 60 min.

Concentration of lipids in the haemolymph

The change in haemolymph lipid concentration in treated and untreated locusts was measured following flight activity and injection of adipokinetic hormones. Haemolymph samples were obtained by pricking the membrane between hindleg and thorax. The hormone was injected in 10 µl distilled water through the intersegmental cuticle between thorax and abdomen. Haemolymph lipid concentration was measured spectrophotometrically using the method described by Zöllner & Kirsch (1962).

Substances tested and controls

1. Neem oil enriched with methanolic extracts of pressed neem seed kernels to a final concentration of 0.075% azadirachtin (prepared at the University Giessen = Neem Giessen)
2. Neem oil enriched with an azadirachtin content of 0.25% (prepared at IITA, Benin = Neem Benin)
3. Pure neem oil pressed from neem seeds (origin: Republic of Niger)
4. Pure palm oil as control (origin: Republic of Nigeria)
5. Adipokinetic hormone (synthetic *Schistocerca* adipokinetic hormone I, Peninsula Laboratories, Belmont, USA).

The neem products used were a gift from Prof. Schmutterer, University of Giessen, FRG, Dipl.-Biol. J. Langewald, IITA, Cotonou, Benin, and Dipl.-Biol. H. Ostermann, Protection des Végétaux, Niamey, Republic of Niger.

Statistical analysis

After confirming that the data were normally distributed, statistical significance was tested by Student's t-test. If not mentioned otherwise, significance was calculated on a 95% level ($P \leq 0.05$).

Results and discussion

Mortality rate and flight activity of *S. gregaria* treated during the resting phase

In initial ground application trials neither the treated population nor the untreated control showed a significant increase in mortality rate during the observation period of 15 days. Nevertheless, two days after application the flight performance of these locusts was tested. The flight distance was measured every five minutes to control the uniform course of the flight activity (Fig. 1).

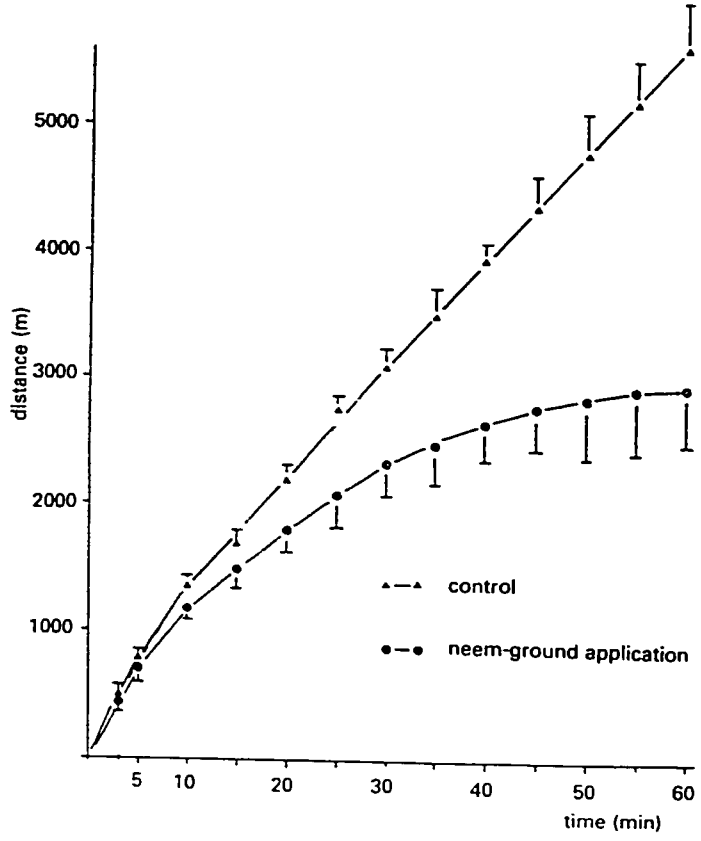

Figure 1. Flight performance of treated and untreated *S. gregaria* (distance in m/h). The data are the mean ± SD of 3 replicates with 6 locusts for each experiment.

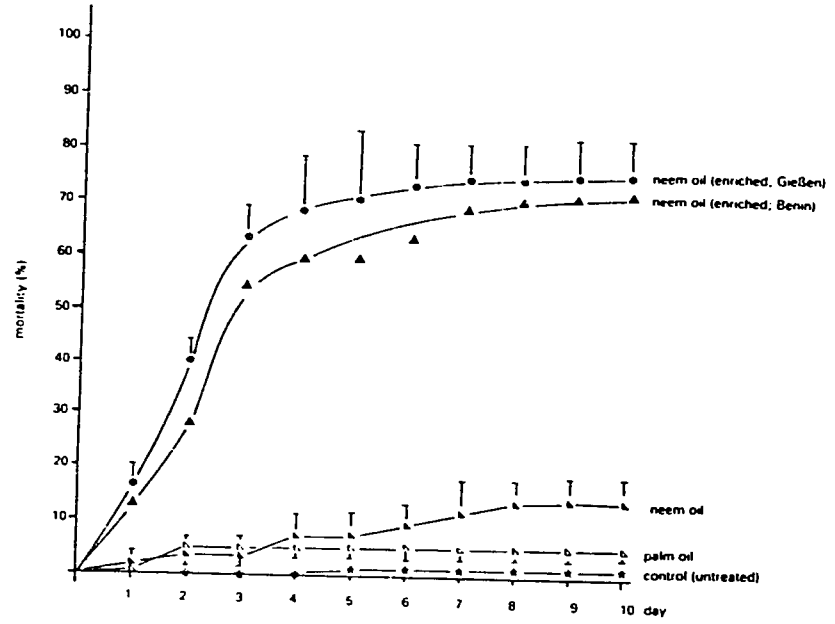

Figure 2. Mortality rate of *S. gregaria* treated during flight activity. The curves demonstrate the increase in mortality expressed as % of the initial population size. The data are the mean ± SD of 4 to 5 replicates for each experiment, except the application of neem Benin (unreplicated).

Although enriched neem oil applied during a resting phase did not affect the life span of *S. gregaria*, it reduced the flight performance by more than 50%. With respect to locust control these results are interesting because they demonstrate that neem oil can influence the migration behaviour of *S. gregaria*. If it is possible to treat locusts in the desert, far from agriculture, reduction of flight activity might be a useful effect because treated locusts may be more susceptible to predators.

This reduction is not sufficient to combat locusts that have reached cultivated areas. In this case, an increase in the mortality rate of the treated locusts is necessary. Therefore neem products were also tested against flying locusts. This technique was attempted for the following two reasons:

1. to produce an effect on *S. gregaria* neem oil has to pass the cuticle but the most sensitive parts of the insect body (such as intersegmental membranes or wing joints) are covered by the wings during resting periods
2. the dispersal of a substance in an insect body is correlated with the metabolic rate. According to the findings of Weis-Fogh (1952), the

metabolic rate of *S. gregaria* increases during flight activity by a factor of about 50 and the effect of neem might therefore be enhanced during flight.

The effect of various neem products and palm oil applied during flight activity on the mortality rate of *S. gregaria* is presented in Fig. 2.

Neem Giessen and neem Benin increased the mortality rate to up to 70% of the initial population within 3 to 4 days and even pure neem oil caused a slightly increased mortality rate. This was in contrast to the natural mortality of the untreated and palm oil controls which remained below 10%. The comparable results of neem Benin and neem Giessen were surprising because these products differ in azadirachtin content by a factor of nearly 3. This may be of importance when developing new neem products since azadirachtin is said to be the most effective neem compound, but also the most difficult component to extract.

The flight performance of neem Benin- and neem Giessen-treated locusts did not differ significantly in the various measurements. Therefore only the flight performance of neem Giessen-treated *S. gregaria* is presented in Fig. 3.

After treatment with neem oil, the flight performance of *S. gregaria* decreased by more than 60% (Fig. 3). The fact that the flight performance improved slightly at day six after spraying does not point to a recovery of the insects but was caused by the fact that by this time only the less contaminated locusts survived (mortality rate more than 70%).

The flight periods were measured for only 30 min, because about 60% of the treated *S. gregaria* did not sustain a continuous flight activity longer than 30 min; 30–40% of the insects had already stopped flying after 10 to 20 min and furthermore, nearly 30% of the locusts treated with enriched neem oil were completely incapable of any flight activity.

The flight performance of *S. gregaria* sprayed with pure neem oil was only slightly affected (data not shown).

Migrating locusts utilize lipids stored in the fat body as fuel for long distance flights. The onset of flight activity is registered by the brain and leads to the secretion of AKH, which are synthesized and stored in the corpora cardiaca. The hormones are released into the haemolymph and transported to the fat body. In this organ the hormones initiate the activation of the lipid mobilizing system. Lipids in the form of fatty acids are secreted into the haemolymph and transported to the flight muscle, where a complete oxidation to water and carbon dioxide yields energy. Therefore the haemolymph lipid profile depends on the physiological state of the insect, and furthermore can be influenced by the injection of synthetic AKH. Thus changes in blood lipid concentration in treated and untreated locusts may be mediated by flight activity or hormone injection and allow conclusions about the mode of action of neem products and the physical capacity of treated and untreated locusts to be drawn. The differences in

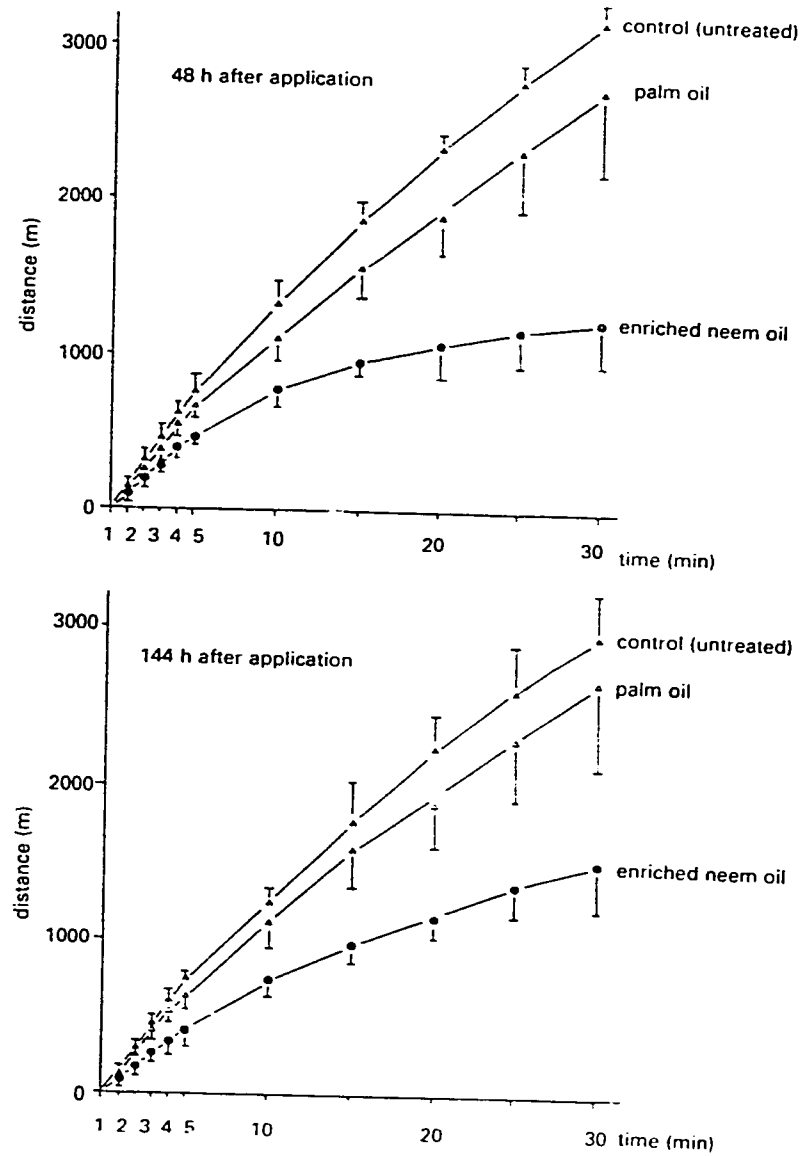

Figure 3. Flight performance of *S. gregaria* 48 h and 144 h after flight treatment. The curves present the flight performance given in m/30 min for *S. gregaria* untreated and treated with enriched neem oil (0.075% azadirachtin) or palm oil. The data are the mean ± SD of 3 to 4 replicates with 6 locusts each.

blood lipid concentration before and after flight or AKH injection of control and neem treated locusts are shown in Fig. 4.

In untreated locusts, flight activity decreased the blood lipid level, whereas the injection of AKH increased the lipid content. The lower lipid concentration resulting from flight activity indicated that the lipids released by the fat body were oxidized immediately to provide the energy needed for this physical activity. The increase in lipids after AKH injection demonstrated the hormone induced activation of the lipid mobilizing system, and the fact that these lipids were not oxidized because the animals were not allowed to fly. In ground-treated locusts comparable observations were made, but the lipid content after flight as well as AKH injection was reduced, indicating that the energy mobilizing system was affected by the neem products. In flight-treated *S. gregaria* a complete disruption of the energy providing system can be observed. Neither the flight activity nor the

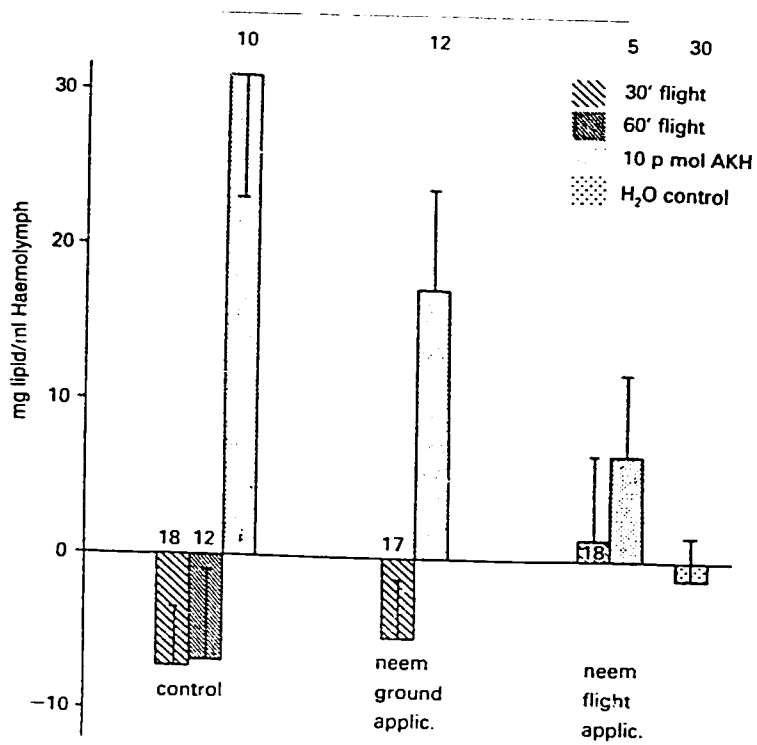

Figure 4. The columns represent the difference in blood lipid concentration before and after flight activity and AKH injection, respectively and were expressed in mg lipid/ml haemolymph. The data are the average ± SD of 5 to 30 estimates for each column, indicated by the number above each column.

AKH injection led to a large change in blood lipid concentration. Therefore one may assume that these locusts were completely unable to provide their flight muscles with the energy necessary for long distance flights.

Conclusions

Ground treatment with enriched neem oil did not affect the mortality rate of *S. gregaria* but decreased the flight performance, and influenced the lipid mobilizing system. The same products applied during flight increased the mortality rate up to 70% and led to a complete inability to sustain flight activity, also induced a disruption of the energy mobilizing system. Flight treatment promises a successful strategy for the use of neem products against *S. gregaria*, but further investigations are necessary. The goals of these investigatons must be oriented toward:

1. varying the formulation to obtain higher concentrations of the effective compounds in neem (to reduce the application volume and to test the feasibility of a ground application)
2. further dose-response experiments involving various application methods in order to minimize the dosage needed
3. a better understanding of the mode of action of the neem products. This must include the identification of the most effective compound in the seed kernel extracts.

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A review of field studies of the environmental impacts of locust/grasshopper control programmes in Africa

P.C. Matteson

Department of Entomology, Iowa State University.

Abstract

The desert environment may be adversely affected by the application of chemical pesticides for desert locust control and microbial pesticides must also be monitored for environmental side effects. Locusts concentrate to feed and reproduce in areas of vegetation which are also richest in non-target species. The microecology of these habitats is very important in determining the effects of pesticides, as is the application method used. The effects on pollinators have caused particular concern and methods have been evolved to study impact on honey bees. Effects on other non-target organisms are best studied by time series analysis, since the large areas and long time scales required for conventional analysis are not usually practical. The results of some preliminary studies on non-target effects of locust control indicate significant effects on terrestrial arthropods, aquatic organisms and birds. More work is needed on methodology and on criteria for judging the acceptability of pesticide side-effects.

Introduction

The plague of desert locust (*Schistocerca gregaria* (Forskål)) that afflicted Africa and the Middle East from 1986–9, coupled with a simultaneous grasshopper outbreak in the West African Sahel, triggered a massive emergency response from the international community. The problem was countered with the same method employed during the last major plague, 25 years previously: insecticide application to acridid swarms and their feeding and breeding sites. Extensive land areas were treated. Nearly 14 million ha in Africa were sprayed for locusts, and insecticides were applied to

additional millions of hectares in the Sahel for grasshopper control (Everts, 1990b; OTA, 1990).

Also, as in the past, little or no environmental monitoring was done in connection with the acridid control campaign. Insufficient data were available to estimate the campaign's impact on non-target species, or for informed decision making on controversial questions such as the relative environmental costs of dieldrin and less persistent chemicals that were proposed as substitutes (Van der Valk, 1988). The assumption that pesticide applications in the desert cause scant or no environmental damage is being questioned. USAID's Programmatic Environmental Assessment of the locust/grasshopper control programme in Africa and the Middle East (TAMS/CICP, 1989) underlined the importance of avoiding treatment of fragile habitats, of developing alternative control methods to replace the current unilateral dependence on synthetic insecticides, and of making environmental monitoring a central element of acridid control activities.

USAID and other agricultural development organizations are acting on those recommendations. Since insecticides are the main locust/grasshopper control method available for Africa in the near future and will probably remain part of integrated control programmes, field researchers have begun to investigate insecticide impact on non-target species. Experimental findings will help to develop safe and selective application guidelines as well as environmental monitoring methods.

Microbial insecticides must undergo scrutiny just as synthetic and botanical ones do. They may harm non-target species which are rare or which are pollinators, scavengers, important food sources for insectivores, or predators and parasitoids of insect pests (Laird *et al.*, 1990). Much of the following applies to entomopathogens, should field tests be required.

Characteristics of locust/grasshopper control campaigns that affect pesticide hazard to non-target species

Certain characteristics of locust/grasshopper control campaigns and the arid environments in which they operate affect the hazard to non-target species. Most of these characteristics increase hazards (de Visscher *et al.*, 1988; Everts, 1990a).

Seasonality

Locusts and grasshoppers are attracted to moist, green habitats to eat and reproduce. In the desert this means oases, wadis with permanent vegetation, or restricted areas where rainfall has produced temporary plant cover and the desert is in bloom for a brief period. These habitats are rich in species taking advantage of favourable conditions for their annual period of activity and reproduction, not only acridids, but other vertebrate and invertebrate herbivores and their predators, as well as human nomads with their livestock. Thus, locust/grasshopper insecticide applications are made when and where they can do the most damage to non-target species.

On the other hand, the extreme seasonal conditions in desert environments, particularly the irregularity of rainfall, have caused native flora and fauna to develop defences against a temporarily unsuitable environment. For example, many organisms are dormant during dry periods, with development and activity triggered by rainfall; however, in some species (including some of the major natural enemies of acridids) only a portion of the population is reactivated at any one time, with delays between emergences lasting months or years (Greathead, 1963). Such adaptations could protect part of non-target arthropod populations from exposure to a small number of insecticide applications (Grant, 1989), and it is important to consider the biologies of non-target organisms when interpreting risk.

Isolation of sensitive habitats

Many rich habitats, particularly oases, permanent springs and temporary pools, are isolated. Ephemeral and non-mobile animal populations are vulnerable to local extinction with poor prospects for recolonization.

Enhanced degradation of insecticides

High UV light intensity and high temperatures promote insecticide degradation, as does increased microbial activity during rainy periods (Grant, 1989). Thus the residual effect of insecticides applied in locust/grasshopper habitats may be much shorter-lived than in temperate zones.

Large scale insecticide application

Treatments generally cover large areas, up to 10 km^2 for locusts and 100+ km^2 for grasshoppers (Everts, 1990b). This slows or even prevents the recovery of non-target species through recolonization. However, prospects for population recovery in savannas are probably relatively good, since even a large treated parcel is surrounded by similar untreated grassland.

Application methods

Equipment used for large scale operations, especially fixed-wing aircraft, decreases the precision of applications and increases environmental risks. Water holes, water sources for treated cropland and other small or dispersed non-target habitats are frequently contaminated.

Insecticide dosages

The insecticide dosages used for locust control are generally higher than dosages applied in other crop protection campaigns. In practice, overdoses are common and can cause dramatic ecological damage.

Repetition of treatments

Repeated insecticide applications multiply the hazard to non-target species and may seriously delay the recovery of populations. A series of applications in a single location is frequently made to control grasshoppers, and sometimes for locust control.

Ecological importance of locusts and grasshoppers

Many vertebrate and invertebrate species use acridids as a food source. This increases the risk of secondary poisoning of insectivores and of sudden significant food deprivation for some non-target species after an insecticide application.

Field research methods for determining insecticide impact on non-target species

Pesticide hazard to a non-target species is evaluated by combining data on pesticide toxicity and exposure, and then interpreting the risk in the light of life history, application patterns, habitat and other factors that will determine the severity of effects and the ability of the population to recover. The epidemiology of entomopathogens may play a significant role where microbial pesticides are concerned. Predatory and parasitic arthropods can disseminate pathogens within host populations, sometimes with a serious negative effect on the beneficials (Vinson, 1990). It is not possible to estimate risk accurately without a field experiment to reflect genuine exposure (Grant, 1988, 1989; Jepson, 1988; Brown, 1989). Preliminary toxicity trials in the laboratory are useful for identifying susceptible non-target groups that should be the focus of field trials.

Honey-bees

Honey-bee (*Apis mellifera* Linnaeus) poisoning by insecticides has become the number one problem for bee-keepers on a worldwide scale (Atkins, 1975; Johansen, 1977), and honey-bees are customarily included in studies of pesticide impact on non-target species. Oomen (1986) and Felton *et al.* (1986) have proposed a sequential evaluation scheme that would start with laboratory screening, continue with cage trials and end with field studies.

Insecticide impact on honey-bees is measured in the field by observing exposed colonies for several days before and after the insecticide application. Queen and brood condition, the number of dead bees and foraging flights at colony entrances, and foraging activity on a treated crop can all be monitored (Gary, 1967; Gary & Mussen, 1984; Gary & Lorenzen, 1989). In addition, caged bees can be exposed to pesticide applications to determine direct effects. Dead bees may be analysed for insecticide residues to confirm cause of death. Experienced local beekeepers who inspect bee colonies before and after the insecticide application can evaluate the probable long-term impact of the insecticide as well as the resulting economic losses.

Other non-target species

Field experiments usually involve periodic sampling to compare changes in target and non-target species numbers or impact (percentage parasitism, predation) between treated and untreated areas before and after pesticide application. Ideally, an experiment would investigate the entire recovery phase of non-target species populations over the total area affected. Since the area affected is normally much larger than the largest possible experimental plots and recovery may require several years, limited experimental budgets and other constraints rarely permit studies of such extent. The failure to distinguish between the experimental and biological dimensions is one of the major flaws in the design and interpretation of pesticide side-effects studies (Jepson, 1989; Everts, 1990a).

Because of the potential for dispersal and redistribution of non-target species between plots, small-plot experiments are generally suitable only for short-term monitoring studies. Season-long investigations may require very large plots (many square kilometers) to be realistic, especially for birds and flying insects. Replication raises the likelihood of observing significant treatment effects, and means that the experimental results can be considered applicable to other similar habitats, not only to the specific plots used (Hurlbert, 1984). However, where large plot sizes are required it can be difficult or impossible to replicate (Sotherton *et al.*, 1988). In that case, a time series analysis is preferred. In order to obtain statistically analysable

results, a period of pretreatment monitoring of all plots is required, as well as post-treatment monitoring (Stewart-Oaten *et al.*, 1986; FAO, 1989).

Birds, mammals, fish and aquatic and terrestrial invertebrates are the non-target groups most frequently observed to determine pesticide side-effects. Terrestrial arthropods, particularly pollinators and beneficial species associated with insect pests, would be the focus of studies on the non-target effects of microbial insecticides (EPA, 1989). Previous work has shown that entomopathogens can harm beneficial insects through direct infection and/or through a reduction in the availability or quality of their food sources. Parasitic Hymenoptera appear to be particularly vulnerable. While entomopathogens are known to reduce prey available to predators, they are decidedly less hazardous to predators than are synthetic broad-spectrum insecticides (Vinson, 1990).

Most arthropod experiments have involved extensive collecting using general sampling methods, and have analysed findings only at order or family level. Yet genus and species level information is usually necessary for a meaningful study. Species within a taxon that have particular ecological and/or economic significance may react much differently to a pesticide application than does the taxon as a whole (Sotherton *et al.*, 1988; Van der Valk, 1990).

To confirm the causal relationship between a pesticide application and an observed effect, at least two of the following observations should be made (FAO, 1989):

- Coincidence of treatment and effect
- Dose-response relationship
- Observed recovery from effect
- Additional information from residue or cholinesterase measurement
- Observations of effects in individual organisms.

Work to date and future directions

Reports have been published recently on initial experiments to determine the ecological impacts of locust/grasshopper insecticides in Africa (Ottesen, 1987; Ottesen & Sømme, 1987; Dynamac, 1988a, 1988b; Müller, 1988, 1990; Pinto *et al.*, 1988; Ottesen *et al.*, 1989; Everts, 1990b). Due to lack of time, resources and/or skilled personnel, few of the experiments followed the ideal experimental guidelines described above. Results are thus of limited applicability, but a general picture of the impact of some commonly used locust/grasshopper insecticides is emerging (Everts, 1990b). Terrestrial arthropods and aquatic animals (particularly crustaceans) are significantly affected, and severe effects on birds have been

observed in some cases. Not nearly enough is yet known to designate 'indicator species' that can simplify the monitoring of pesticide impact (Everts, 1983), or to design practical environmental monitoring methods for insecticide application teams.

Consistent with their recent emphasis on integrated pest management and sustainable agriculture, international development organizations are continuing to increase their attention to the ecological effects of pesticides. Further work is needed to improve experimental methodology for studies of insecticide effect on non-target arthropods. Possibilities of supplementing field trials with laboratory or semi-laboratory approaches should be investigated. Statistical methods need to be improved for analysing time series data and indirect phenomena such as insecticide-induced pest resurgence, and for detecting small changes in non-target arthropod populations that exhibit wide natural fluctuations in population density. Appropriate time scales for observations must be determined and applied. There also remain considerable problems with interpreting field studies and establishing indicators of environmental quality (Sotherton *et al.*, 1988). This is especially true for Africa, where the taxonomic status and ecology of so many arthropod species are unknown.

Finally, there is the question of criteria for judging the acceptability of pesticide side-effects. What limits should be set on honey-bee mortality and reduction of populations of threatened or endangered species? Are perturbations in beneficial insect populations acceptable if detectable for only one season? Two seasons? These and similar questions merit careful investigation. Policymakers depend on scientists for defining key issues for proposing research and for advice on adequate environmental protection standards.

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Environmental impact and safety of fungal biocontrol agents

M.S. Goettel & D.L. Johnson

Agriculture Canada Research Station, Lethbridge, Alberta.

Abstract

Compared with chemical insecticides, fungal microbial control agents offer, among other advantages, a method of insect pest control that has a very narrow host range, can usually be integrated with other biocontrol agents, may provide prolonged control by establishment in the host population, and is also biodegradable. A brief review of the potential environmental impact and safety of candidate fungal control agents is presented. There are indications that these organisms pose only a minimal risk to humans, domestic animals and wildlife. The wide host ranges of some species have caused concern regarding safety to non-target invertebrates. Results of a laboratory study on the safety of a grasshopper isolate of *Beauveria bassiana* Balsamo (Vuillemin) to alfalfa leafcutter bees are reported. No significant increase in bee mortality resulted from treatment of alfalfa with *B. bassiana* conidia. However, the limitations in our knowledge of fungal epizootiology make it impossible to extrapolate from such data to the field situation. Continuous monitoring during field use after preliminary laboratory and field safety testing is recommended to ensure safety to non-target invertebrates.

Introduction

Compared with chemical insecticides, fungal microbial control agents offer, among other advantages, a method of insect pest control that has a very narrow host range, can usually be integrated with other biocontrol agents, may provide prolonged control by establishment in the host population, and is also biodegradable. The best documented cases of detrimental

environmental impact of fungal biocontrol agents are indirect effects on the predators and parasitoids of the target pest through host depletion (Goettel *et al.*, 1990). In certain instances, fungi may also adversely affect certain non-target invertebrates directly including predators and parasitoids (Goettel *et al.*, 1990; Vinson, 1990). In general, it is not possible to reduce a pest population without also affecting another component of the ecosystem. For instance, by depleting or even reducing a host population, it is inevitable that the host's predators and parasites will be adversely affected. In such situations, it is imperative that an integrated pest management programme be put into effect that would integrate fungal control with other available control methods.

Safety

A review of present knowledge of potential fungal control agents indicates that these organisms pose only a minimal risk to humans (Siegel & Shadduck, 1990), domestic animals and wildlife (Saik *et al.*, 1990), and non-target invertebrates (Goettel *et al.*, 1990). Nevertheless, the general consensus is that since fungi may pose inherent risks they should be regulated. Most guidelines for registration of entomopathogenic fungi require laboratory testing for infectivity to non-target organisms including mammals (Hall *et al.*, 1982; Aizawa, 1990; Betz *et al.*, 1990; Kandybin & Smirnov, 1990; Quinlan, 1990).

The potential hazards from fungal microbial control agents to higher animals including man, centre on direct exposure to high levels of inoculum. These hazards include toxicity, allergenicity, and infection, and are generally associated with improper handling and application of the product. As pointed out by Prior (1990), these do not generally pose new problems since the same types of hazards are present and dealt with in the use of chemical pesticides.

Host range and specificity

The most likely environmental risk of fungal microbial agents is that non-target invertebrates may be infected and killed. A 'safe' microbial insecticide is essentially one with a restricted host range under the conditions of use. Some fungal species are highly specific and pose a minimal threat to non-target invertebrates. For instance, *Entomophaga grylli* (Fresenius) Batko is restricted to Orthoptera even to the point of species specificity of pathotypes (MacLeod, 1963). Some other entomopathogenic fungi have very wide host ranges. For instance, *Beauveria bassiana* Balsamo (Vuillemin) has been recorded as infecting over 700 species of arthropods (Li, 1987).

However, laboratory studies have shown that different isolates of the same species with a wide host range are generally most virulent to the host from which they were first isolated (Goettel *et al.*, 1990). Prior (1990) points out that there are some exceptions; certain strains possess high pathogenicity to previously unencountered hosts.

Fungi with wide host ranges are frequently more specific under field conditions, especially during epizootics (Goettel *et al.*, 1990). There are several reports of fungi attacking only one host even though closely related species are present. Such resistance is thought to occur as a result of the complex biotic and abiotic interactions which occur in the field. Furthermore, some insects can be infected readily in the laboratory by fungi not known to attack them naturally.

Host specificity needs much further investigation, both from the point of view of the range of arthropods infected, and from that of the biotic and abiotic factors or interactions responsible for its restriction or extension. In view of the complexities of the field situation, great caution should be exercised when attempting to extrapolate laboratory results.

Safety testing

Even though the value of laboratory results on non-target susceptibility to fungal agents is dubious as far as establishing safety in the field is concerned, most requirements for registration and even limited field experimentation require laboratory safety testing to non-target invertebrates. Such requirements usually include the honey-bee (*Apis mellifera* Linnaeus) as a test organism, but laboratory testing of entomopathogenic fungi against social insects such as the honey-bee poses unique problems. For instance, Vandenberg (1990) demonstrated that a strain of *B. bassiana* caused mycosis among honey-bees treated with very high doses of conidia, although the result was obscured by the fact that mortality was not significantly different from the control. If a pollinator species must be included in safety testing, more suitable candidates should be used. The sustained viability of caged leafcutter bees would make these bees better candidates for pollinator safety testing requirements because caged honey-bees do not survive well.

Safety of *B. bassiana* to the alfalfa leafcutter bee

The alfalfa leafcutter bee, *Megachile rotundata* (Fabricius), is used extensively in North America for pollination of seed alfalfa (*Medicago sativa*). Although no records of *B. bassiana* infecting leafcutter bees in nature have been reported (Goettel *et al.*, 1990), recently Goerzen *et al.* (1990) demonstrated that prepupae were susceptible to the fungus. Caution is

therefore warranted if applications include direct exposure of bee colonies or foragers.

We tested the effects to adult leafcutter bees of *B. bassiana* applied directly onto alfalfa. The *B. bassiana* isolate used is being developed by Mycotech Corporation, Butte, MT, for control of grasshoppers (Johnson *et al.*, these proceedings). The fungus was supplied as a dry conidial preparation containing starch. Conidial viability was determined to be 60%. Three 2 l pots of flowering alfalfa cv. Beaver, were placed in each of 10 cages, 1.32 m^2. The plants in five cages were sprayed with a conidial suspension in water at a rate of 1×10^9 viable conidia per cage. This is equivalent to 1×10^{13} conidia/ha, the target application rate for grasshopper control. Uniformity of spray application was verified through the use of water sensitive paper. Twenty-five male and 15 female leafcutter bees were added to each cage 12 h after the alfalfa had been sprayed. Equivalent numbers were added to five paired cages containing unsprayed alfalfa. Mortality of the bees was monitored daily for 14 days. Samples of dead bees were surface-sterilized with 40% ethanol and held in a chamber at 100% RH and room temperature to encourage fungus growth on the cadavers. At the termination of the experiment, a census was made of a number of bees remaining in each cage, and the initial cage counts were adjusted to account for a few bees that escaped.

After 14 days, there was no female mortality and male mortality averaged 9% in the treated cages. There were no significant differences in mortality between treatment and control bees; however, all surface-sterilized cadavers from treated cages produced *B. bassiana* hyphae and conidia within three days, indicating that the bees were infected with the fungus.

Conclusions

With few exceptions, fungi are generally safe and do not pose any serious threat to higher animals. The wide host ranges of some species have caused some concern regarding safety to non-target invertebrates, and this has hindered their field testing. Current recommendations for registration include laboratory infection tests on selected non-target invertebrates including bees. However, the limitations in our knowledge of fungal specificity and how it relates to epizootiology make it impossible to extrapolate such data to the field situation. Based on present knowledge of fungal epizootiology, a better approach for ensuring long-term safety to invertebrates would be continuous monitoring during and after field application following preliminary laboratory and field safety testing. It is imperative that excessive safety and registration requirements do not

hinder the development of fungal insecticides, which will be generally much safer than the chemicals used at present.

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Panel discussion: precis

Dr Bateman opened the discussion by asking whether replications can be achieved repeating the same experiment in sequence. Dr Johnson replied that this could be done but that the problems lie in designing replication in time and in its analysis. Asked about statistical treatment, Dr Johnson recommended using time in the model, for example using pre-treatment counts and time as co-variates or analysing weekly time-points separately.

Dr Prior wanted to know what sort of co-variate should be used. Dr Johnson suggested the pre-treatment population density. Each co-variate only loses one degree of freedom, and age and body weight can also be used as co-variates and tested in the model.

Dr Oomen asked how we should approach the problem of testing a slow acting agent such as a fungus. Migration in and out of the plots occurs during the incubation period; how big should the plots be and can we decrease the number of replicates as we increase the plot size? Dr Johnson suggested the marking and recapture of insects to allow following the insects and said that a good understanding of the ecology of each grasshopper species is essential. Migration can be treated as a variable, always remembering that treatment may affect migration. It is essential to concentrate on the individual insects and always maintain a rigorous approach.

Dr Matteson suggested time series analysis as a substitute for replication. To do this a long period of sampling before and after treatment is required. Dr Johnson agreed that replication in time can be a valuable approach, however the method is still controversial and should not be a substitute for replication in space in our experiments. Besides the statistical problems there are also logical problems in treating time as a variable.

Dr Cardwell indicated that it was necessary to know the time x treatment interaction and that sequential analysis would not give this interaction. Dr Johnson agreed that this may be better modelled by other means since times are not randomized.

Dr Mouhim asked about the population level of grasshoppers in trials in Mali with *Beauveria bassiana* and was told that the density varied between blocks but was about 15–22 individuals/m^2, but certainly not low.

Dr Magor asked whether migration could be ascertained from GIS data and Dr Johnson said that he was trying to build this element into the model and is sure that it can be done.

Dr Auld asked Dr Keller what the concentration of skimmed milk used in his formulation was. The reply was 1%. Dr Milner asked what the spore application rate was for *Beauveria* using cereal bait at a dose of 200 kg/ha. The spore production was not known, but the dose applied was 100 kg/ha which will be reduced to 30–50 kg/ha. The spore production from each infected larva was approximately 10^9 per larva.

Dr Bateman asked Dr Lobo Lima what sort of oil had been used in Cape Verde and how it was mixed with water. Dr Lobo Lima and Dr Henry said that it was a vegetable oil produced by Mycotech (Mycotech Corporation, Butte, MT) where the emulsion was produced by shaking oil and water together, and some problems with separation were experienced.

Dr Lomer wanted to verify the plot size used and was informed that it was 0.5 ha.

Dr Prior first expressed his gratitude to Dr Nasseh for presenting the rather disappointing results for *B. bassiana* and wanted to know why it hadn't worked. Was it perhaps the temperature? Dr Nasseh indicated that they had experienced a range of temperatures from 12°C to 42°C in the desert with a humidity between 15% away from the plant and 35% next to the plant. He thought that the combination of temperature and humidity might be a factor. Dr Prior then asked if the viability of the formulation had been checked prior to usage and both Dr Nasseh and Dr Wilps indicated that it had not.

Dr Oomen asked Dr Matteson how she could judge whether possible effects found in field trials were acceptable or not. Dr Matteson's reply was that these factors were almost always political. As an example, supposing you found 50% of an important parasitoid killed by a pesticide, the problem in interpreting the effect could be, will this level of mortality cause pest resurgence or affect the crop? What losses are people willing to tolerate in exchange for the benefits of using pesticides? None of these decisions are simple.

Dr Knausenberger asked Dr Matteson whether most of the recent work had been done in recession breeding sites. Are there possibilities for several teams in different areas to support work already being done? How relevant is the work done so far for ecotoxicological studies of remote sites which are poorly known but which may be important breeding

sites? Dr Matteson replied that until now the assumption made has been that we are only treating the desert so why worry? The excellent work done by PRIFAS has shown conclusively that locusts need green plants to eat and humidity in order to breed – so they need the oases and wadis and other temporary green areas. Other invertebrates and vertebrates, including birds and other predators, also come to these places, as do nomads with their herds. Insecticide applications are inevitably made at a time and place where their impact is maximized. In an environment where there are irregular rainfall and harsh conditions the native species have developed strategies against harsh conditions. In the diapause staggered batches of eggs can mature months or even years apart. It is essential to understand the biology of species in order to assess the impact of potential solutions. With large application sites and the use of aeroplanes to apply treatments, the potential for contaminating water holes or the very sensitive environment of the desert is enormous. We must also remember that the locust is important in the diet of many local animals.

Dr Bateman asked whether safety tests for mycoinsecticides should be done on a species to species basis, to which Dr Goettel replied that he thought testing should be examined case by case. The scientist should provide necessary data on strain formulation and the authorities should decide on the basis of these data. For example, conidia do not reproduce in rabbit brain – but might they not do so if in an oil based formulation? The ramifications are endless. Safety regulators themselves have to be careful, since a poor decision could influence their jobs, but one of the problems in this area is that they don't know much about pathogens.

Dr Bateman also wished to know Dr Goettel's own opinion as to how regulation should be done and he replied that in his view low risk pathogenic species can be identified in the laboratory and then closely monitored under controlled conditions over a long period in the field. If there is an indication of an effect on mammals or humans then further tests will obviously be required, but it is difficult to justify the safety testing of each strain on mammals simply because each one has a slightly different specificity from an insect species.

Dr Evans then asked whether Dr Goettel was referring to Canadian, EPA or international regulations. Dr Goettel maintained that there is turmoil in regulation everywhere. For example, in certain states in the USA there are rules about testing from EPA, APHIS and the state to fulfill. Dr Evans felt that things were not quite so gloomy and that there are indications that officials of the EPA and USDA in the USA are trying to improve testing on a species to species basis, partly in order to save money, for example, *Bacillus thuringiensis* species, the first codling moth virus from California, or the granulosis virus, are test cases in this area. Dr Goettel mentioned that the EPA has just recently insisted on the re-registration of every *B. thuringiensis* strain. Despite the fact that they have all been in use

for years, they all have to be tested again using 'good laboratory practice'.

Dr Goettel asked why neem oil had been chosen to mix with the spores, and Dr Nasseh replied that as mixing *Beauveria bassiana* with mineral oil had produced no conclusive results, they tried neem oil and obtained a slight mortality.

Dr Goettel was then asked, when using commercialized spore formulations, how often one should return to the original strain in order not to lose any virulence? He replied that it depends on the fungus – some lose their virulence after two cultures and others reproduce a sub-culture for years without losing virulence. It is important to deposit useful isolates in a recognised culture collection in liquid nitrogen so that the strain is available for years to come. It may be important to consider the substrate as an important factor in affecting the virulence of a strain.

Dr Lomer took this opportunity to point out that all the field experiments which had negative results using *Beauveria bassiana* had used the Mycotech formulation which had been specifically selected for productivity and not for virulence. Dr Nasseh agreed that this was the case, but Dr Goettel pointed out that results in the laboratory had been satisfactory and perhaps the answer lay instead in further work on the host range. The session chairman, Dr Johnson, pointed out that isolates can vary in their sensitivity to ultra-violet light and this might be one of the reasons why the fungus killed in the laboratory but not in the field. Dr Nasseh added that those desert locusts in humid conditions were affected by the fungus.

Mr Fousséni added a human dimension to the discussion, saying that farmers have been battling with the locust problem for centuries and have been using insecticides for 50 years. When did the panel think that the farmer might be able to use this ecologically sound product? The chairman passed the question to Dr Goettel who felt that because the technology is so new and innovative, it is difficult to give a precise idea – it could be very soon or many years. There are so many hypotheses still to test, results must be consistent and this is not the case at the moment where products work in the laboratory but not in the field. More work is needed on UV protectants. If a heavy concentration of fungal spores is applied in the field it might affect insect parasites, parasitoids and predators which all fluctuate with the pest. Natural epizootics of microbial pathogens occur very rarely and do not occur without the right environmental conditions. If we want to take a short cut to induce an epizootic artificially we have to do more than provide sufficient infective inoculum to start the epizootic. There is a big difference between microbial control and control by parasitoids

In reply to Mr Fousséni's original question Dr Prior stated that experiments on biological control of locusts have gone from unproven theory to proven kill within the short time of 18 months and that this can be consolidated within the next 18 months. He was of the opinion that the technology should be available after a further five years and that he would be surprised

if it weren't possible within that time, so maybe in seven years from now the product would be available to the farmer.

Dr Johnson referred back to Dr Goettel's comments saying that in his opinion there was more to an epizootic than the lack of spores in the right conditions since it hasn't yet proved possible to trigger an epizootic as such. Dr Goettel responded that the interaction between the environment, the biology of the insect and the inoculum was still insufficiently understood. Dr Johnson maintained that what was needed was a host for the spore population since it seemed clear that the missing element was not a release of spore mass. Dr Goettel suggested that what was being done was the use of a microbial pathogen as an insectide to induce massive mortality and that we have to iron out the problems between the laboratory and the field first. Dr Johnson felt that maybe we were trying to measure something precisely without actually knowing what that something is.

Dr Gutierrez intervened at this point to ask whether it was not probable that environmental factors affect pathogens on the plants and plant pathologists have modelled these interactions, so it might be worth surveying what has already been done. Did Dr Goettel know of any specific examples of the effect of pathogens on population dynamics of species? Usually insects, host plant and parasitoids were present at the same time, but here they were not. Dr Goettel quoted *E. grylli* as a complex example where attempts are being made to model the epizootic. Dr Gutierrez wanted to know whether it is possible to model the effects using rainfall patterns to obtain the mortality without knowing the dynamics. Would a lot of inoculum be needed? Dr Goettel wondered whether conidia need humidity and if a rapid epizootic could be induced using a specific amount of conidia, for example as in aphids.

Section VI

Closing Session

Discussion: precis

Dr Knausenberger commented that the objectives of the meeting, i.e. to discuss achievements and future objectives, have been reached, with a concrete development in the form of international links between locust and grasshopper groups and he hoped that these links will grow stronger, as there is much expertise in North America and Europe to be tapped. There should be more work on fully involving African experts and including their initiatives and interests in research. It is important to involve Africans in field trials this season as GTZ and other groups, including the IITA/IIBC/DFPV axis, are already doing. There are many people in research systems all over the Sahel who are close to the problem and the country representatives should inform their people that there is a new tool on the horizon and a specific agent to be tried out this season. It is important that they identify the colleagues who can be of most help. In talking with Mme Ba-Diallo he had already got a list of two to three people per country who should be involved as entomologists or pathologists. The key is involvement. There is a long horizon for improvement. At the moment *Metarhizium* seems to be the most promising, but other solutions will come. The potential for *Beauveria* is high and we are working on a strain enhancement in Canada and the USA. Who knows what the entomopox virus research in Cape Verde will produce? We have achieved and surpassed the purpose of the workshop and he saw this programme as a viable and continuing area of activity and this is the message he would take back to USAID. The tensions during the workshop were helpful as there was no ill-will, rather a creative tension which is helpful in debates and insights from different approaches, which is good. He hoped that everyone feels the same – it may be discouraging for those who are involved in this kind of activity for the first time. The motivation for the workshop to arrive at an alternative to

chemical pesticides is definitely going in the right direction, it may take us ten years but there may also be middle ways – the home cottage industry for example, as with producing neem, maybe local production of *Beauveria* and *Metarhizium* with bran using appropriate technology to involve the creativity of the individual. We should maintain this decentralized activity, spread knowledge and wealth and put it in the hands of the people who can then find their own solution to the problem.

Dr Henry presented some ideas on training. In order to help the evaluation of microbial pathogens of grasshoppers and other insects, a course in insect pathology is proposed which would concentrate on grasshoppers and locusts and would be a three month intensive course.

It could either be held at Montana State University or in collaboration with IIBC/IITA in Benin. The obvious advantage to holding the course in Benin is that it would be less expensive, while Montana has a well established grasshopper laboratory with a staff of 11 scientists, five of whom are grasshopper pathologists. There is also extensive literature available.

Candidates should be of university level with a BSc or equivalent and the course would be taught in French and English for African and Asian participants.

Syllabus:

1. microscopy including electron microscopy
2. histology
3. abiotic diseases
4. biotic diseases
   — bacteria
   — fungi
   — viruses
   — protozoa
5. disease epizootiology
   — natural
   — applied
6. bioassay protocol
   — laboratory
   — field – plot design
7. reporting results.

If there is enough interest a formal programme will be developed and the necessary funds sought.

Dr Smit asked whether Dr Henry thought that people educated in the techniques for experiments and trials would be able to do the experiments themselves. What about the great need for equipment? Dr Henry agreed that there was a real problem regarding equipment in many locations, but that for isolating and characterizing most microbial pathogens all that was needed was a good compound microscope. His intention was not to make

instant experts, but to inform and communicate. Dr Smit stated that in the experience of DFPV in training field and laboratory technicians with a medium level of education, it is necessary to train them well, even though they are not graduates, as they are the backbone of much authoritative work in the difficult discipline of crop protection. Dr Henry agreed that technicians are important but suggested that this was not an appropriate level for Montana State University and that it might be better handled at local level. He also maintained that another benefit to training at a higher level was that the trainees can then train or help to train technicians themselves.

Dr Lomer wanted to put on record that insect pathology is not difficult and that any entomologist at the end of two months of microscope training should know enough pathology to do field experiments.

Dr Bani wanted the meeting to think very carefully about the African context, given that most African countries are poor and it is important that the training be given to those who will benefit most and can then pass on that benefit. For example, at the end of secondary level schooling in the Congo a student knows nothing about microscopes, fungi or viruses and would not be able to cope with training at the level described by Dr Henry, which would then not have the desired impact. It is better to train someone who has the right background and who can then explain and pass on his training to others.

Mr Maiga agreed with Dr Bani and Dr Henry, saying that the problem in Africa was that there are many entomologists but no phytopathologists or entomopathologists and that Africans have been saying this for a long time. We are looking for some ideas to help our people. Another delegate said that the training service of the plant protection services was the only way to perpetuate the work on biological control.

Mr Wodageneh said that all training is important and that it should be given priority. One of the most important aspects is the proper selection of candidates for training – and this must be done by consultation with officials at national level and by interviews with potential candidates. In this way officials value the training and we get the proper candidates. By building up this trust with officials we have a high success rate in retaining trainees in the jobs for which they were trained – 80% are still in place after 6–7 years. The level of education must be carefully screened – many people of a relatively low level of education make excellent field workers and technicians given the proper training, but there must also be appropriate training available to those with a higher level of education. We need to train more assistants than leaders. With some knowledge of entomology and pathology and an elementary knowledge of how to use a microscope the students can, with a little training use their sound basis to get a programme started.

Dr Herren agreed that it is essential to train at both technical and

academic levels and said that in his view extension and research go hand in hand. He said that we need help from the national representatives because this is a new area for Africa and all levels of training are needed. We must start with academic training so that these people, once trained, can go home and teach or do research in their own right.

Dr Smit reiterated that DFPV train technicians for nine Sahelian countries and they have sent questionnaires out to these countries to try to assess the training needs. They find that they are getting fewer and fewer candidates for training and need to know why this is. By July they will hopefully have analysed the questionnaires. He emphasized that training technicians is important since you soon get enough high level people – but of course, both are important.

Mr Gallédou said that little had been heard about biological control by insect predators of grasshoppers. These are important, and there are interesting studies of egg pod parasites, for example. Might there also be other possibilities for biological control of other pests such as the mealybug of date palm? He mentioned that he was pleased to have come to the workshop and was delighted with what he had heard, as he regards the use of chemical insecticides as an attack on traditional lifestyles. Biological control of locusts could improve the farmer's lot and it would be a good thing to associate the farmer with such control measures. On the whole it was a better outlook for the environment.

Mr Hammond said that when trying to build a biological control capacity in Africa, we must be careful to use an integrated pest control approach. The technicians should only have a short training since they are not the people who will make research decisions. Technicians have other functions, are not specialized and often may not even be available to do the work they have been trained for. There should be an emphasis on training people with a basic university education to do a specific job. They should be able to handle biological control including parasites and microbial pathogens. Not all the available money for training should be spent on training technicians.

Dr Lomer affirmed that support was needed from the top to advance this type of research.

Dr Mushi remarked that plant protection experts were in short supply, and there are no experts in biological control. His experience suggests that short courses can be useful for technicians. To make a breakthrough in this field we need people who can influence the policy makers. Co-operation between the USA, Europe and Africans can convince donors. At this stage he requested that funds be made available to implement training recommendations. The first priority is high level manpower development and the second is the training of technicians for once they are trained they can, in turn, train local staff.

An American delegate said that we must work with the existing institutions at both levels. Advanced methodology in insect pathology can be given to Africans – as an example to strengthen the existing ICIPE programme through collaboration.

Mr Saizonou recommended training specialists in biological control and ecology along with technicians who will be in contact with the farmers. He recommended a programme which would include both extension and research aspects.

Dr Nkouka supported Dr Herren and Mr Wodageneh, saying that an army cannot function with only soldiers or only generals. There are important human resources in Africa involved in the national programmes and they should be clearly identified. There are many people working in national programmes who are not in the right places. Everyone wants to go on a course and when they return, many want to do everything except that which they learnt on the course. It is essential that the managers of national programmes go and look at their people and give their opinions of what is needed. It is often the case that the people who go to scientific conferences are not scientists but administrators who cannot properly benefit by the experience. Africa does not start from zero in this matter for there are many laboratories at national institutes of agronomy and in universities. We must look at the human resources – some university lecturers may know about microbiology. We are not about to remake the world but must reinforce what already exists. If it is necessary then we can send people abroad, but it does not matter where the basic techniques are acquired as long as they are learned and used. The research and techniques should be placed in their work situation and therefore in Africa. We must check what is already available.

Dr Lomer agreed that it was essential to identify available resources and this was supported by Dr Knausenberger who suggested an inventory of manpower such as DFPV already does for the Sahel. He went on to say that he looked to all the participants from UNDP, FAO, and IITA, for advice in this area. The exchanges had been very valuable in discussing the kind of training needed and the kind of people to train and he suggested setting up a committee to assess the training question. He noted the efforts in this area of PRIFAS, IITA, and DFPV, and the strong presence of the GTZ programme in Africa. There are many experts in biological control using pathogens and there is a network already involved in training – Dr Smit, Mr Wodageneh and others, who should meet and come up with a strategy.

Dr Herren recommended that Dr Nkouka be involved since FAO sponsor IITA's training UNDP/FAO programme. The consensus was that Drs Smit, Nkouka, Lomer and Wodageneh should meet within the next few days to devise a strategy with which to tackle the extensive research, technical and academic training needed, taking into account what is already available.

Dr Lomer thanked the interpreters Claire Lord, Oscar Hounvou, and Carol Moudachirou, the administrative staff, especially Jacob Quaye, Samson Nyampong, and Leo Sossou, and the drivers, for their help in running the workshop.

Dr Roberts concluded the meeting by thanking Dr Lomer and his wife for their part in organizing the workshop, despite their having been in Benin for only three months.

Acronyms and abbreviations

a.i.	active ingredient
ADV	average droplet volume
AGRHYMET	Agronomie Hydrologie Météorologie (CILSS)
AKH	adipokinetic hormones
ALRC	Anti-Locust Research Centre
APHIS	Animal and Plant Health Inspection Service (USDA)
ARSEF	Agricultural Research Service Collection of Entomopathogenic Fungi (USDA)
BBA	Biologische Bundesanstalt für Land- und Forstwirtschaft
BCP	Biological Control Programme (IITA)
CABI	CAB International
cc	corpora cardiaca
CDA	controlled doplet application
CEC	Commission of European Communities
CG	Management Committee (DFPV)
CHEF	contour-clamped homogeneous electric field
CIDA	Canadian International Development Agency
CILBA	Complexe International de Lutte Biologique Agropolis
CILSS	Comité Inter-Etats pour la Lutte contre la Sécheresse dans le Sahel
CINA	Centre National de Lutte Antiacridienne
CIRAD	Centre de Coopération Internationale en Recherche Agronomique pour le Développement
CNLA	Centre National de Lutte Anti-acridienne (Morocco)

CNRS	Centre National de la Recherche Scientifique (Montpellier)
COPR	Centre for Overseas Pest Research (UK)
CSIRO	Commonwealth Scientific and Industrial Research Organization (Australia)
CSP	Scientific and Pedagogical Committee (DFPV)
DFPV	Département pour la Formation en Protection des Végétaux
DGIS	Netherlands Directorate General for International Cooperation
DLCOEA	Desert Locust Control Organization for Eastern Afr
DPP	Directorate for Plant Protection (Senegal)
DTX	destruxins
EC	emulsifiable concentrate
EDTA	ethylenediaminetetra-acetic acid
ENSAM	Ecole Nationale Supérieure d'Agronomie de Montpellier
EPA	Environmental Protection Agency
FAO	Food and Agriculture Organization (United Nations
GERDAT	Département de Gestion, Recherche, Documentatio et Appui Technique
GHIPM	Grasshopper Integrated Pest Management
GTZ	Gesellschaft für Technische Zusammenarbeit (Germany)
HAN	heavy aromatic naphtha
I.U.	international unit
IAPSC	Inter-African Phytosanitary Council (OAU)
ICIPE	International Centre of Insect Physiology and Ecology (Kenya)
ICTZ	Inter-Tropical Convergence Zone
IGR	Insect Growth Regulator
IIBC	International Institute of Biological Control
IITA	International Institute of Tropical Agriculture
IMI	International Mycological Institute
INIA	National Institute for Agricultural Research (Cape Verde)
INRA	Institut National de la Recherche Agronomique
INSAH	Sahelian Institute (Bamako)
IPARC	International Pesticide Application Research Centre (Imperial College of Science, Technology and Medicine, London)
IPDR	Institute for Practical Rural Development (Niger)
IRLCOCSA	International Red Locust Control Organization for Central and Southern Africa

LD	lethal dose
LGBCP	Locust and Grasshopper Biological Control Programme
MPV	*Melanolpus* entomopoxvirus
NDVI	Normalized Difference Vegetation Index
NPV	nuclear polyhedrosis virus
NRI	Natural Resources Institute (ODA)
OAU	Organization for African Unity
OCLALAV	Office Centrale pour la Lutte Anti-acridienne et Anti-avaire
ODA	Overseas Development Administration (UK)
OPV	*Oedaleus* entomopoxvirus
ORSTOM	Office de la Recherche Scientifique et Technique Outre-Mer
OTA	Office of Technology Assessment (US Congress)
PAGE	polyacrimide gel electrophoresis
PCR	polymerase chain reaction
PFGE	pulsed field gel electrophoresis
PIB	polyhedral inclusion body
PPS	Plant Protection Service (Mali)
PRIFAS	Acridologie Opérationnelle – Ecoforce Internationale Programme de Recherche Interdisciplinaire Français sur les Acridiens du Sahel
PVP	polyvinyl pyrolidine
RAPD	randomly amplified polymorphic DNA
RESADOC	Sahelian Documentation Network (INSAH)
RFLP	restriction fragment length polymorphism
SAS	Surveillance des Acridiens du Sahel
SDAYE	Sabouraud's dextrose agar with yeast extract
SDS	sodium dodecyl sulphate
SNPV	Service Nationale de la Protection des Végétaux
SPC	Surveillance des Pathogènes des Criquets (DFPV)
TDRI	Tropical Development Research Centre
TRAIS	Tropical Agriculture Information Service (NRI)
TSPV	Training Course for Crop Protection Technicians (DFPV)
ULV	ultra-low volume
UNDP	United Nations Development Programme
USAID	United States Agency for International Development
USDA	United States Department of Agriculture
UV	ultra-violet
VAD	volume average diameter
VMD	volume mean diameter

Addresses of delegates

Mr A. Ali Abdalla
c/o Dr W.G. Mohamed
Head of Locust and Grasshopper
Control Unit
Plant Protection Department
PO Box 8192 Elamarat
Knartoum
Sudan
Tlx: 240565 GTZ

Mr L. de Abreu
Crop Protection Service
c/o USDA-APHIS
Guinea-Bissau

Dr J.B. Ackonor
Entomology Division
Cocoa Research Institute
Private Mail Bag
Kotoka International Airport
Accra
Ghana

Dr N. Aho
Directeur Général Adjoint
Centre Béninois de Recherche
Scientifique et Technique
BP 03-1665
Cotonou
Bénin
Tel: (229) 32 21 63

Ms G.O. Akpokodje
Department of Agricultural Biology
University of Ibadan
Oyo State
Nigeria

Dr B.A. Auld
Agriculture Research and Veterinary
Centre
Forest Road
Orange 2800
New South Wales
Australia
Fax: (61) 63 636799
Tel: (61) 63 636700

Dr D. Ba-Diallo
CILSS-INSAH-UCTR/PV
BP 1530
Bamako
Mali
Fax: (227) 73 33 37
Tel: (227) 73 21 81
Tlx: 55 45 DFPVNI

Dr J.W. Bahana
International Red Locust Control
Organisation for Central and Southern
Africa
PO Box 240252
Ndola

Zambia
Tel: (260) 2 614584/5; 615684
Tlx: ZA 30072

Dr G. Bani
Direction Générale de la Recherche
Scientifique
BP 2499 Brazzaville
Congo

Prof. S. El Bashir
ICIPE
PO Box 30772
Nairobi
Kenya
Fax: (254) 2 80 33 60
Tel: (254) 2 80 25 01/3/9
Tlx: 22053/25066

Dr R.P. Bateman
International Institute of Biological
Control
Silwood Park
Buckhurst Road
Ascot
Berks SL5 7TA
UK
Fax: (44) 344 872901
Tel: (44) 344 87299
Tlx: 93121022555

Mr E. Baumann
Ciba-Geigy
Agriculture Division
AG 714
CH-4002 Basel
Switzerland
Tel: (41) 61 697 71 60
Fax: (41) 61 697 39 70
Tlx: 926 355 CG CH

Mr M. Baumgart
Projet Neem GTZ
Institut für Phytophathologie und
angewandte Zoologie
Universität Giessen
Ludwigstrasse 23
6300 Giessen
Germany
Tel: (49) 641 74596
Fax: (49) 641 74596

Mr J. Brito
CX Postal 128
Praia
Cabo Verde Islands
Fax: c/o (238) 612020
Tel: (238) 61570

Dr K. Cardwell
International Institute of Tropical
Agriculture
Oyo Road
PMB 5320 Ibadan
Nigeria
Fax: (234) 611896/(229) 301466
Tlx: 31417

Dr C.W. Castleton
Area Director
USDA-APHIS
L'Ambassade des Etats Unis
BP 1712 Abidjan
Côte d'Ivoire 01
Fax: (225) 22 32 59
Tel: (225) 21 09 79
Tlx: 23660 AMEMBCI

Dr A.K. Charnley
School of Biological Sciences
University of Bath
Claverton Down
Bath
Avon BA2 7AY
UK
Fax: (44) 225 826779
Tel: (44) 225 826826
Tlx: 449097

Dr J. Clarkson
School of Biological Sciences
University of Bath
Claverton Down
Bath
Avon BA2 7AY
UK
Fax: (44) 225 826779
Tel: (44) 225 826826
Tlx: 449097

Mr A. Coffi
Chef, Division Protection des Végétaux
BP 648
Cotonou
Bénin

Dr G.L. Cunningham
USDA APHIS-PPQ
Grasshopper IPM Project
3380 Americana Terrace
Boise
Idaho 8370
USA
Fax: 208 384 3920
Tel: 208 334 9644

Prof. V. Delucchi
ETH
Institut für Pflanzenwissenschaften
Bereich Phytomedizin
Clausiusstrasse 21
8092 Zurich
Switzerland
Fax: (41) 262 2546
Tel: (41) 256 22 11/256 39 30

Dr J.S. Evans
1034 Spring Drive
Boulder
Colorado 80303
USA
Fax: (1) 303 673 0133
Tel: (1) 403 499 1413
Tlx: 023 40 18928

Mr A. Fousséni
Direction du Service de la Protection
des Végétaux
BP 1263
Cacavali, Lomé
Togo

Dr. H. Franzen
ATSAF
German Council for Tropical and
Subtropical Agricultural Research
Hans Böckler Strasse 5
D-5300 Bonn 3
Germany
Fax: (49) 228 4001 311

Dr K.S. Fischer
DDG Research
International Institute of Tropical
Agriculture
Oyo Road
PMB 5320 Ibadan
Nigeria
Fax: (234) 611896/ (229) 301466
Tlx: 31417

Mr le Gall
ORSTOM
Brazzaville
Congo

Mr T. Galledou
Bureau OASIS/DA
BP 180 Nouakchott
Mauritania
Tel: 54769
Tlx: 5867 MTN

Mr S. Ganbobo
Directeur de la Protection des Végétaux
Minstere de l'Agriculture
BP 323
Niamey
Niger

Dr L. van der Geest
Department of Pure & Applied Biology
University of Amsterdam
Kruislaan 302
1098 SM Amsterdam
Netherlands
Fax: (31) 205 257754
Tel: (31) 205 257740
Tlx: 16460

Mr S. Glodjinon
Ciba-Geigy Services S.A.
BP 8123
Cotonou
Bénin

Dr M. Goettel
Agriculture Canada
Lethbridge Research Station
P.O. Box 3000 Main
Lethbridge
Alberta T1J 4B1
Canada
Fax: (1) 403 382 3156
Tel: (1) 403 327 4561

Dr D.J. Greathead
International Institute of Biological
Control
Silwood Park
Buckhurst Road

Ascot
Berks SL5 7TA
UK
Fax: (44) 344 872901
Tel: (44) 344 872999
Tlx: 93121022555

Dr D. Greenland
CAB International
Wallingford
Oxon OX10 8DE
UK
Fax: (44) 491 33508
Tel: (44) 491 32111
Tlx: 847964

Prof. A.P. Gutierrez
Division of Biological Control
University of California, Berkeley
1050 San Pablo Avenue
Albany CA 94706
USA
Fax: (1) 415 642 08 75
Tel: (1) 415 642 71 91
Tlx: 910 366 7114

Mr J. Gwinner
GTZ/MGBPP
PO Box 49
Muzuzu
Malawi
Fax: 332 396
Tlx: 0222

Dr W.N.O. Hammond
West and Central Africa Regional
Coordinator
IITA Biological Control Programme
Biological Control Centre for Africa
BP 08 0932
Bénin
Fax: (229) 301466
Tel: (229) 301994/360188
Tlx: 5329 Itaben

Ms K. Hell
Service Protection des Végétaux
DAGRI BP 58
Porto Novo
Bénin
Tel: (229) 21 32 90/93

Dr J.E. Henry
Montana State University
USDA Rangeland
Bozeman, MT 59717
USA
Fax: (1) 406 9946462

Dr H.R. Herren
Programme Director
IITA Biological Control Programme
Biological Control Centre for Africa
BP 08-0932 Cotonou
Bénin
Fax: (229) 301466
Tel: (229) 301994/360188
Tlx. 5329 Itaben

Son Excellence M.P. Houtoundji
Ministre de l'Education
BP 348 Cotonou
Bénin
Tel: (229) 31 06 81/19 91/16 93

Dr D.L. Johnson
Agriculture Canada
Lethbridge Research Station
P.O. Box 3000 Main
Lethbridge
Alberta T1J 4B1
Canada
Fax: 403 382 3156
Tel: 403 327 4561

Dr K. Jones
ODA Natural Resources Institute
Central Avenue
Chatham Maritime
Chatham
Kent ME4 4TB
UK
Fax: (44) 634 880066/77
Tlx: 263907/8

Dr S. Keller
Federal Research Station for Agronomy
Postfach 8046
Zürich
Switzerland
Fax: (41) 3777 201
Tel: (41) 3777 111

Captain R.A. Kitenda
Director for Operations
DLCO-EA
PO Box 30023
Nairobi
Kenya
Fax: (254) 2 50 51 37
Tel: (254) 505517

Dr W. Knausenberger
US Agency for International Development
Africa Bureau AFR/TR/ANR
Washington, DC 20523-1515
USA
Fax: (1) 703 235 3805
Tel: (1) 703 235 3826

Mr A. Kogo-Salaou
GTZ Service d'Administration des Projets
BP 10814
Niamey
Niger
Fax: (227) 73 26 29
Tel: (227) 72 25 51
Tlx: 5410 GTZ Ni

Mr S. Koné
Sockim / Ciba-Geiby
Division Agriculture
BP Abidjan
Côte d'Ivoire 01
Fax: (225) 35 08 00
Tel: (225) 35 56 25
Tlx: 43215

Ir C. Kooyman
DFPV
BP 12625
Niamey
Niger
Fax: (227) 73 22 37
Tel: (227) 73 21 81
Tlx: 5545 DFPVNI

Mr S. Krall
Plant Protection & International Agricultural Research
GTZ, Dag-Hammarskjöld-Weg 1-2
BP 5180
D-6236 Eschborn 1
Germany
Fax: (49) 6196 797150- 791115
Tlx: 4075010

Mr. J. Langewald
IITA Biological Control Programme
Biological Control Centre for Africa
BP 08 0932
Cotonou
Bénin
Fax: (229) 30 14 66
Tel: (229) 30 19 94/30 14 66
Tlx: 5329 Itaben

Dr M.H. Launois-Luong
CIRAD-PRIFAS
BP 5035
Avenue du Val de Montferrand
34032 Montpellier Cedex 1
France
Fax: (33) 67 41 09 58
Tel: (33) 67 61 58 00
Tlx: 480762F

Mr C. Lawani
Chief Service Protection des Végétaux
DAGRI B.P. 58
Porto Novo
Bénin

Dr M.L. Lobo Lima
President of INIA
CX Postal 128
Praia
Cape Verde Islands
Tel: (238) 61570
Fax: c/o (238) 612020

Dr C.J. Lomer
IIBC/IITA-BCP Locust Project
IITA Biological Control Programme
Biological Control Centre for Africa
BP 08 0932 Cotonou
Bénin
Fax: (229) 301466
Tel: (229) 301994/360188
Tlx: 5329 Itaben

Mr W. Luhanga
Lunyangwa Research Station
PO Box 59
Muzuzu
Malawi
Fax: 332396
Tlx: 0222

Dr J.I. Magor
Natural Resources Institute
Central Avenue
Chatham Maritime
Chatham
Kent ME4 4TB
UK
Fax: (44) 634 880066/77
Tlx: 263907/8 LDNG mark
attn Magor at NRI
Tel: 0634 883270

Mr B. Maiga
Service de la Protection des Végétaux
BP 1560
Bamako
Mali

Dr P. Matteson
Department of Entomology
Iowa State University
Ames
Iowa 50011-3140
USA
Fax: (515) 294 8027
Tel: (515) 294 4916

Dr G.A. Matthews
Director, IPARC
Imperial College of Science, Medicine & Technology
Silwood Park
Buckhurst Road
Ascot
Berkshire SL5 7TA
UK
Fax: (44) 344 294450/294339
Tel: (44) 344 23911
Tlx: 929484

Mr A.L. Mbiele
Secrétaire Scientifique
Conseil Phytosanitaire Interafricain
OUA
BP 4170 Nlongkak
Yaoundé
Cameroun
Tel: (237) 22 25 28
Tlx: 8460

Mr A. Meier
Ciba-Geigy
Agriculture Division
CH-4002 Basle
Switzerland
Tel: (41) 61 697 71 60
Fax: (41) 61 697 39 70
Tlx: 926 355 CG CH

Dr A. Mendonça
UFAL Campus Rio Largo
Caixa Postal 672
57001 Maceio, Al
Brazil
Fax: (55) 82 221 7876
Tlx: (55) 82 157 XPMCB BR

Dr R. Milner
CSIRO
Division of Entomology
PO Box 1700
Canberra ACT 2601
Australia
Fax: (61) 6 246 4000
Tel: (61) 6 246 4911
Tlx: AA 62309

Dr D. Moore
International Institute of Biological Control
Silwood Park
Buckhurst Road
Ascot
Berks SL5 7TA
UK
Fax: (44) 344 872901
Tel: (44) 344 872999
Tlx: 93121022555

Mr A. Mouhim
Centre National de la Lutte Anti-Acridienne
BP 125 Inezgane
Morocco
Fax: (212) 83 05 70
Tel: (212) 83 12 21/83 16 76
Tlx: 81741

Mr A. Mushi
Assistant Commissioner
Plant Protection Services
Ministry of Agriculture & Livestock Development

PO Box 9071
Dar es Salaam
Fax: (255) 51 38 428

Dr O.M. Nasseh
Plant Protection & International Agricultural Research
GTZ, Dag-Hammarskjöld-Weg 1-2
BP 5180
D-6236 Eschborn 1
Germany
Fax: (49) 6196 797150/791115
Tel: (49) 6196 790
Tlx: 4075010

Mr A.B. Ndiaye
OCLALAV
BP 1066
Dakar
Sénégal
Fax: (221) 32 04 87
Tel: (221) 32 34 79/32 80
Tlx: 61 238 OLA

Dr P. Neuenschwander
IITA Biological Control Programme
Biological Control Centre for Africa
BP 08-0932 Cotonou
Bénin
Fax: (229) 301466
Tel: (229) 301994/360188
Tlx: 5329 Itaben

Mr A. Niassy
Chef du Laboratoire d'Entomologie
Direction de la Protection des Végétaux
BP 20054 Thiaroye
Dakar
Sénégal
Fax: (221) 34 42 90
Tel: (221) 34 42 94

Dr N. Nkouka
OUA/ISTRC
Conseil Phytosanitaire Interafricain
BP 4170 Nlongkak
Yaoundé
Cameroun
Tel: (237) 20 25 28
Tlx: 8460 KN

Dr P.A. Oomen
Plant Protection Service
PO Box 9102
6700 HC Wageningen
Netherlands
Fax: (31) 8370 21701
Tlx: 45163 PD NL

Dr A. Paraïso
Locust Project
IITA Biological Control Programme
Biological Control Centre for Africa
BP 08-0932 Cotonou
Bénin
Fax: (229) 301466
Tel: (229) 301994/360188
Tlx: 5329 Itaben

His Excellency W.M. Pridun
Ambassador of Austria
Embassy of Austria
Olosa Street
Victoria Island
Lagos
Nigeria
Tlx: 21463 OENIGNG

Dr W.J. Primeau
Anglophone Africa Branch
CIDA
Promenade du Portage
Hull
Quebec
Canada K1A 0G4
Fax: (1) 403 819 953 6379
Tel: (1) 403 819 997 0984
Tlx: 053 4140

Dr C. Prior
International Institute of Biological Control
Silwood Park
Buckhurst Road
Ascot
Berks SL5 7TA
UK
Fax: (44) 344 872901
Tel: (44) 344 872999
Tlx: 93121022555

Dr S.K. Raina
ICIPE

PO Box 30772
Nairobi
Kenya
Fax: (254) 2 80 33 60
Tel: (254) 2 80 25 01/3/9
Tlx: 22053/25066

Dr D. Roberts
Insect Pathology Resource Center
Boyce Thompson Institute for Plant Research
Cornell University
Tower Road
Ithaca
New York 14853-1801
USA
Fax: (1) 607 254 1242
Tel: (1) 607 254 1352

Mr S. Saizonou
Service Protection des Végétaux
DAGRI BP 58
Porto Novo
Bénin

Mr S. Sanyang
Department of Agricultural Services
Yundum Agricultural Station
Ministry of Agriculture
The Gambia
Tel: 82758/95420/95377

Mr E. Sayoum
International Institute of Biological Control
Silwood Park
Buckhurst Road
Ascot
Berks SL5 7TA
UK
Fax: (44) 344 872901
Tel: (44) 344 872999
Tlx: 93121022555

Dr F. Schulleri
BP 1073
Porto Novo
Bénin

Mr P. Shah
International Institute of Biological Control
Silwood Park
Buckhurst Road
Ascot
Berks SL5 7TA
UK
Fax: (44) 344 872901
Tel: (44) 344 872999
Tlx: 93121022555

Mr R. da Silva
PNUD
Cotonou
Bénin
Tel: (229) 32 30 45/46/79

Ir J.J. Smit
DFPV
BP 12625
Niamey
Niger
Fax: (227) 73 22 37
Tel: (227) 73 21 81
Tlx: 55 45 DFPVNI

Mr G. Stachel
Ministry of Foreign Affairs
Austrian Development Copperation
Ballhausplatz 3
A-1014 Vienna
Austria
Fax: (43) 222 5354530
Tel: (43) 222 9370295

Dr W.M. Steele
international Institute of Biological Control
Silwood Park
Buckhurst Road
Ascot
Berks SL5 7TA
UK
Fax: (44) 344 872901
Tel: (44) 344 872999
Tlx: 93121022555

Dr D. Streett
USDA/ARS
Montana State University
Rangeland Insect Laboratory
Bozeman MT 59717
USA
Fax: (1) 406 9946462

Dr L. White
Africa International Service
USDA-APHIS
American Embassy
BP 1712 Abidjan
Côte d'Ivoire 01
Fax: (225) 21 32 59

Dr H. Wilps
Forstzoologisches Institut
Universität Freiburg
D-7800 Freiburg
Germany
Fax: (49) 761 203 2496
Tel: (49) 761 203 2496

Dr A. Wodageneh
FAO/BCP Principal Training Officer
IITA Biological Control Programme
Biological Control Centre for Africa
BP 08 0932 Cotonou
Bénin
Fax: (229) 30 14 66
Tel: (229) 30 19 94/35 01 88
Tlx: 5329 Itaben

Dr B. Zelazny
Institute for Biological Pest Control
Biologische Bundesanstalt
Heinrichstrasse 243
D-6100 Darmstadt
Germany
Fax: (49) 6151 42 25 02
Tel: (49) 6151 44061

Dr M. Zweigert
GTZ/BCP Regional Coordinator
IITA Biological Control Programme
Biological Control Centre for Africa
BP 08 0932 Cotonou
Bénin
Fax: (229) 30 14 66
Tel: (229) 30 19 94/36 01 88
Tlx: 5329 Itaben

Author index

Abdalla, A. 97
Auld, B.A. 219
Bahana, J.W. 57
Bani, G. 79
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Brader, L. ix
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Brito, J.M. 287
Byaruhanga, E.K. 57
Charnley, A.K. 181
Clarkson, J.M. 191
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Cunningham, G.L. 21
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Kooyman, C. 8, 208
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Lobo Lima, M.L. 287
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Matteson, P.C. 347
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Mendonça, A.F. 239
Milner, R.J. 200
Mouhim, A. 38
Nasseh, O. 44, 311, 337
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