

BIOLOGICAL CONTROL OF LOCUSTS AND GRASSHOPPERS

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■ Abstract Control of grasshoppers and locusts has traditionally relied on synthetic insecticides, and for emergency situations this is unlikely to change. However, a growing awareness of the environmental issues associated with acridid control as well as the high costs of emergency control are expanding the demand for biological control. In particular, preventive, integrated control strategies with early interventions will reduce the financial and environmental costs associated with large-scale plague treatments. The recent development of effective oil formulations of *Metarhizium anisopliae* spores in Africa, Australia, and Brazil opens new possibilities for environmentally safe control operations. *Metarhizium* biopesticide kills 70%–90% of treated locusts within 14–20 days, with no measurable impact on nontarget organisms. An integrated pest management strategy, with an emphasis on the use of *Metarhizium*, that incorporates rational use of chemical pesticides with biological options such as the microsporidian *Nosema locustae* and the hymenopteran egg parasitoids *Scelio* spp., has become a realistic option.

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INTRODUCTION

Locusts and grasshoppers (Orthoptera: Acrididae) represent perhaps the most conspicuous of all insect pests and are abundant insects of dry grassland and desert. When populations of these insects build up, certain species exhibit gregarious and migratory behavior, leading to the formation of spectacular swarms. From their mention in the Bible to current media reports, these locust plagues attract public attention in a way that no other insects do; the image of a flying swarm of locusts from the desert descending onto crops never fails to stir the human conscience.

In the majority of cases, national authorities have adequate capacity to conduct preventive control measures, controlling outbreaks at an early stage through the use of chemical pesticides. In countries such as Argentina, Australia, China, Niger, and South Africa, populations of locusts and grasshoppers are monitored and treated as soon as outbreaks threaten. When pests cross national borders, internationally coordinated operations are necessary; we discuss this more complex situation in relation to the desert locust (*Schistocerca gregaria* Forskål) below. Plagues develop only when control efforts break down, or political or natural disasters prevent access to breeding areas, and interventions do not start early enough. Control failures and plague development have occurred with the desert locust in the Red Sea basin in 1986 and 1992 (162, 163), with the migratory locust *Locusta migratoria capitata* (Sauss) in Madagascar in 1995 (189), and with the

Italian locust *Calliptamus italicus* L. in Kazakhstan in 1997 (189). Once plagues develop, curative insecticide applications become necessary over wide areas, with associated financial and environmental costs that are far in excess of the cost of preventive control.

In this review we give an overview of the current status of control options against locusts and grasshoppers and the increase in environmental awareness and political issues associated with locust control. We examine developments in biological control over the last decade, with a particular focus on the development of biological pesticides based on oil formulations of fungal spores. Finally, we discuss how biological control options could be incorporated into integrated pest management (IPM) strategies, and what further research and development work is necessary to implement such IPM strategies.

CURRENT SITUATION: Chemical Control

Locusts were first recognized as a serious agricultural problem by British authorities in Iraq in the 1920s (152, as cited in 186a). A series of international locust conferences took place during the 1930s, from which arose the Anti-Locust Research Centre, subsequently renamed the Centre for Overseas Pest Research. The Centre for Overseas Pest Research handed over responsibility for desert locust control coordination to the Food and Agriculture Organization (FAO) of the United Nations in 1953 (186). The Anti-Locust Research Centre, established in 1945, developed the use of chemical pesticides against locusts, selecting dieldrin as the most effective and economical control agent because of its long persistence (13). Use of dieldrin allowed the treatment of barrier strips; migrating hopper bands would cross these strips and accumulate a lethal dose. The Anti-Locust Research Center also pioneered the aerial application of low doses of dieldrin as a fine droplet cloud to control desert locust swarms (154), one of the most efficient applications of chemical pesticides known (124).

An increasing awareness of the negative environmental impact of organochlorine pesticides has led to restriction of their use to certain limited public health applications. Organophosphate, carbamate, and pyrethroid pesticides replaced dieldrin for locust and grasshopper control, but these could not be tested against African locusts because populations of those insects were in recession. The upsurge in the populations of desert, migratory, and red (*Nomadacris septemfasciata* Serville) locusts in the early 1980s, after more than 30 years of recession, found the authorities unprepared (162, 138). Not only had trained teams dispersed, but the most effective pesticide, dieldrin, was no longer available for use. The substitute organophosphate pesticides, such as fenitrothion and malathion, had shorter environmental persistence and were often repeatedly applied as blanket treatments over large areas. Ironically, such treatments may have caused greater environmental damage than the organochlorine treatments they were designed to replace (153).

Most modern control operations rely on safer organophosphates such as fenitrothion, carbamates such as bendiocarb, pyrethroids such as deltamethrin and lambda-cyhalothrin, fipronil, and insect growth regulators such as dimilin and triflumuron (47).

POLITICAL AND ECONOMIC ISSUES

In attempting to draw up a concise overview of the political and economic issues associated with locust and grasshopper attacks and the economic benefits of different treatment options, we need to consider the following points:

1. Locust plagues are essentially unpredictable; the locust swarm may fly across desert areas into the sea, attack subsistence millet fields, or invade high-value crops. Therefore, not only is the development and movement of the swarm population unpredictable, but also the value of the damage is even more unpredictable and difficult to estimate (80, 93). Nevertheless, the unpredictable risk could be reduced by containing the pest outbreak at its source.
2. The total cash value of the crop damage may be modest by most standards (71, 95), but even modest amounts of total damage may be very severe in some localities and cause disruptions in the local economy (37). In farming systems where >90% of the crops are produced for the personal subsistence of farmers, the use of simple cost-benefit ratios based on the market value of the crops can be questioned. Food aid has sometimes been proposed as an alternative to treatments, but such aid brings its own external costs in terms of the disruption of local food-production systems (138).
3. Most entomological cost-benefit analysis formats rely on the benefit that accrues to the farmer carrying out the control treatments (35a). This formula can be difficult to apply in the case of a migratory pest (168). Indeed, for highly mobile species such as the desert or brown locust, the burden of control may fall on one country whereas the benefits may be accrued somewhere else completely.
4. More than half of pest control costs are usually met by external donors who may have their own objectives (21). In addition, the nature of donor funding for locust control is such that funds are normally considered as one-off disaster funds, released for plague treatment, but not for preventive treatments. This can lead countries to exaggerate infestations so that emergency funds can be used for routine control (96).
5. Because of public pressure or confounded interests of decision makers, governments may conduct control operations irrespective of any expected economic benefit. The environmental impact of such treatments is discussed below.

6. Even where control is justified and necessary, local environmental and economic factors may place restrictions on the type and nature of control operations. For example, use of conventional chemical insecticides may be inappropriate in areas of organic livestock production and national parks and may create pressure for alternative technologies.

Recent studies by the World Bank in Madagascar (189) address some of these concerns. Wright (190) estimated a benefit:cost ratio of between 23:1 and 29:1 for chemical control operations against the Australian plague locust, *Chortoicetes terminifera* Walker.

We can conclude from these observations that locusts will be controlled, irrespective of the costs and benefits. Furthermore, (a) preventive locust control is cheaper and less environmentally damaging than plague control; (b) accurate estimates of the benefits of control are unlikely to be possible in Africa; (c) preventive control will reduce risks of outbreaks and concomitant plague control costs but continuous low-level donor support is needed, even when locust populations are low and the immediate risks minimal. The difficulty in maintaining donor and government support for long-term preventive control programs (and hence maintaining the skilled knowledge base and infrastructure that this brings) is one of the factors that leads to the exacerbation of problems in outbreak situations.

ENVIRONMENTAL ISSUES

Environmental issues arising from the standard use of chemical pesticides against locusts and grasshoppers include the impact on operators, other people, livestock, birds, other terrestrial vertebrates (especially lizards), aquatic organisms (fish and invertebrates), and terrestrial arthropods, including the natural enemies of locusts and grasshoppers, as well as pollution issues, contamination of groundwater and wells, and disposal of surplus pesticide stocks (45). Several publications deal with the state of our knowledge when the alarm was first raised (14, 150), and much useful research has been conducted since then. Murphy et al (134) reviewed the toxicities of commonly used pesticides and found that in 45%–55% of the records, the chemicals gave mortality rates >90% in nontarget species. Initially, desert environments were viewed as fragile per se (e.g. 123); more recently, attempts have been made to define which particular environments are most at risk. In general, ephemeral aquatic habitats are especially vulnerable, particularly if used by migratory birds as feeding and resting areas.

Few attempts have been made to quantify the external costs associated with grasshopper and locust control; Houndekon & DeGroote (64) estimated veterinary, health, and disposal costs and found a small but significant value for these externalities.

Numerous studies relate the negative impact of chemical pesticides on nontarget organisms. Peveling et al (144) showed that in Madagascar even single applications

of fenitrothion, which has shorter environmental persistence, caused long-lasting population declines among epigaeal collembolans. van der Valk et al (111, 183) summarized the impact of insecticide treatments on terrestrial arthropods, particularly natural enemies of acridids, and discussed the possibility that insecticide applications may have increased the impact of grasshoppers in the Sahel (184).

With the available information on the selectivity of chemical pesticides and the areas particularly at risk, it should be possible to design pesticide use so that environmental damage is minimized while control objectives are achieved. For instance, Martin et al (122) investigated the impact of treating grassland with the pyrethroid deltamethrin to control grasshoppers on the availability of insects, primarily grasshoppers, as a food source for grassland songbirds. Levels of control of 90% were economically effective while allowing persistence of sufficient numbers of grasshoppers to allow survival of nestlings and successful fledging. Similarly, the use of low levels of insecticides with known low avian toxicity, such as carbaryl in wheat bran bait (85), has been shown to result in reductions of ~70%, which allows survival of posttreatment populations of 1–5 grasshoppers per square meter. Such levels are well below the economic threshold, but are above the levels required for survival of insectivorous grassland birds. A slightly different approach that focuses on reduction of the total area treated is proposed by Schell & Lockwood (156). The long persistence of both insect growth regulator chemicals and fipronil means that they can be used to treat barrier strips in much the same way as dieldrin was used. In this way, the total dose per hectare is much reduced, as is the impact on nontarget organisms (5, 180). Despite these advances, there remains scope for the use of biological control.

MOVE TOWARDS INTEGRATED PEST MANAGEMENT

Increasing awareness of the economic and environmental cost of failure to prevent locust swarms is leading to a call for a more integrated approach to locust and grasshopper control (72, 80). Although some experts have advised treatment of developing swarms as the most cost-effective option (171), a preventive, strategic approach is now more widely accepted (162). In general, we can identify three essential steps in developing an IPM approach: (a) exploring novel, environmentally benign locust-control technologies, (b) evaluating novel and existing control technologies for their environmental impact and efficacy, and (c) implementing novel and existing technologies with improved forecasting and monitoring in an integrated context. Several national programs are working along these lines. The Australian Plague Locust Commission in Australia, the Agriculture and Agri-food Canada Research Branch in Canada, the Plant Protection Research Institute in South Africa, the Department of Agriculture's Agricultural Research Service in the United States, and the regional plant protection services of the Ministry of Agriculture in Spain are all developing environmentally benign, cost-effective, integrated outbreak-prevention strategies.

Migratory locust species frequently cross national borders, and a complex of coordinating bodies has been established in Africa. The International Red Locust Control Organization for Central and Southern Africa is responsible for control of the red locust *N. septemfasciata* in central and southern Africa. The South African authorities control the brown locust (*Locustana pardalina* Walker) within their national borders and have succeeded in preventing outbreaks for >50 years. The responsibilities of the existing control structures for control of desert locust and migratory locust (the Desert Locust Control Organization for East Africa, and OCLALAV, the French acronym for the organization responsible for control of acridids and migratory birds in West Africa), have to a large extent been replaced by the FAO Emergency Prevention Scheme for transboundary pests and diseases (46). The FAO Emergency Prevention Scheme project coordinates the activities of the national programs in the Red Sea basin desert locust recession area and incorporates recent progress into geographic information systems (GIS), modeling, biological control, and environmental impact evaluation in an integrated preventive control scheme.

A comprehensive inventory of different technology development and environmental impact assessment projects appears in the proceedings of a 1995 meeting held in Bamako, Mali (94).

ACRIDID POPULATION DYNAMICS

It is beyond the scope of this article to present detailed descriptions of population dynamic studies for the diverse range of acridids that can attain pest status throughout the world. Instead, we attempt a brief summary of the important common factors to highlight the nature of the problem and the current status of our knowledge.

Grasshoppers and locusts generally have very high reproductive rates and are able to respond to favorable climatic conditions with rapid population increases. For some species, such as the desert locust, developmental times can be relatively short (and get even shorter following gregarization at high densities) and breeding continuous. Species such as the brown locust and the Senegalese grasshopper *Oedaleus senegalensis* Krauss (12) may have two or three generations within a short season. Many species, such as the Sahelian grasshoppers *Hieroglyphus daganensis* Krauss and *Kraussaria angulifera* (Krauss) (146, 159), have just one generation per year. Common to most is the limitation imposed by abiotic factors, principally the available moisture for egg eclosion and the development of food plants. When these conditions are satisfied, population growth can be rapid. Under less favorable conditions, eggs may remain in diapause in the soil. The eggs of several species are able to survive 2 or 3 years, which allows multiple generations to emerge at one time. The net effect of these factors is that populations tend to be highly variable over time and space, with the potential for rapid development of significant populations. Adding to this variability is the role of biotic factors, such as predators,

parasites, and diseases. Ecological studies on many species have shown that a suite of natural enemies can cause significant mortality at different life stages, and for a range of species there is evidence that population growth is being curtailed at certain times through the action of natural enemies (188). In general, however, mortality from natural enemies does not provide a reliable check to population growth.

Over a longer term, populations are affected by man's agricultural activities, when cultivation destroys the locusts' oviposition sites. The African migratory locust, *L. migratoria migratoria* (Reiche & Fairmaire), has almost disappeared as an agricultural pest in West Africa owing to the increased cultivation of the Niger flood plains. Conversely, in Kazakhstan and Russia, the recent outbreaks of the Italian locust and other species appear to have arisen as a consequence of declining cultivation [the impact of agricultural practices on the Italian locust in Kazakhstan has been studied by Chetyrkina (34)]. The same is true for recent increases in the Moroccan locust, *Dociostaurus maroccanus* Thunberg, in southern Italy.

Various theoretical models have been proposed that combine the abiotic and biotic factors (32, 35, 54, 79) involved in acridid population dynamics. Considerable effort has also been invested in developing practical population dynamic models; although these have been reasonably successful in some cases, for African locusts theoretical models so far are less reliable than practical experience in predicting outbreaks. Progress has been made in describing the habitats and food preferences of the desert locust (38), and the *Schistocerca* Warning Management System GIS is being developed by FAO to enable data coordination (36). The factors determining locust migration are complex and variable, and, given the current state of knowledge, it is not possible to predict when locusts will invade a particular patch of suitable vegetation, nor what the consequences of such an invasion will be.

BIOLOGICAL CONTROL

Considerations of acridid population dynamics are useful in evaluating the potential for biological control. Classical biological control refers to an inoculative introduction of an agent not previously present; in cases where this represents a new association between an effective biological control agent and a pest, it is referred to as a neoclassical biological control (110). Inoculative augmentation refers to the application of an indigenous agent to enhance subsequent buildup in the biocontrol agent population, whereas inundative augmentation refers to the mass application of an agent with the primary objective of high initial kill. In both economic and ecological terms, a classical biological control agent that becomes established and exerts a controlling influence on a pest over an indefinite time period is the ideal control agent.

Many of these biocontrol approaches are applicable to locust and grasshopper control but their potential has been underestimated in the past because of the emphasis on chemical control. Most grasshoppers and locusts are indigenous to their particular environment, so the prospects for classical biological control would not

appear promising. Similarly, rates of acridid population growth and movement appear to exceed those of their natural enemies, and normally the impact of pathogens on populations is minimal. However, the examination of these contraindications in more detail reveals several windows of opportunity. The egg stage is vulnerable to parasitoid attack. Given the high rates of efficacy of some oligophagous *Scelio* spp. in Australia (3), there may be potential for new associations that have not been adequately explored; we discuss this possibility further below.

Arthropod natural enemies of the mobile stages of acridids only build up late in the plague cycle. If the pest habitat has been treated with chemical pesticides, this buildup may be further delayed. It is possible that treatment with a more selective control agent would permit a more rapid buildup in the natural enemy population. Both in this context, and in their own right as stand-alone pest control agents, we can consider the use of pest-specific microorganisms (entomopathogens) as inundative, inoculative, and classical biocontrol agents. The use of entomopathogens as control agents is referred to as microbial control; when the entomopathogenic microbe is mass-produced and formulated, we can refer to it as a biological pesticide (biopesticide) or mycopesticide (when the microbe is a fungus). An emerging theoretical framework for the role of entomopathogens as biological pesticides in IPM (49, 173, 178) leads us to suppose that, if we could find ways to manipulate pathogen populations, we could have a lasting impact on pest populations and exploit their specificity to allow a full role for arthropod natural enemies, all for a minimal environmental impact. Most acridids appear to be quite susceptible to pathogens and normally evade them by preferring dry habitats and moving on to new habitats. Goettel & Johnson (50) provide an overview of the pathogens that affect acridids, including bacteria, viruses, nematodes, microsporidia, and fungi. The first microbial control agent developed for acridid control was *Nosema locustae* Canning (phylum Microspora: Microsporidia: Microsporidae; 82); but a demand for more rapid speed of kill led to the development of fungi capable of penetrating insect cuticle.

DEVELOPMENT OF FUNGAL SPORE FORMULATIONS AS MYCOPESTICIDES

Indigenous deuteromycete fungi have considerable potential as microbial control agents because they are genetically stable and can be produced cheaply in large quantities. Formulating the spores in oil should avoid the normal requirement of such fungi for high humidity during the infection process, and, as the infection penetrates the cuticle, the formulation can be used as a contact insecticide. These considerations formed the conceptual basis for the project Lutte Biologique contre les Locustes et Sauteriaux (LUBILOSA) (149; also see acknowledgments). The LUBILOSA project brought together a multidisciplinary team, which was successful in developing the first effective mycopesticide for locust control; we focus on project results before moving on to consider other developments.

Selection of Pathogen

Many factors need to be taken into account in selecting a pathogen for development as a biopesticide, including its presence in the environment and precedents for successful prior use of similar microbes. Its virulence needs to be checked and one particular isolate selected through a series of bioassays. Further tests check mammalian toxicity, genetic stability, productivity, spore stability, tolerance to UV, heat, and other environmental stresses, field efficacy, safety for nontarget organisms, environmental impact, and recycling (75).

Before 1989, only ~30 isolates of hyphomycete fungi from Orthoptera were available in international culture collections. Currently, the number is nearer 300; about 180 were collected by the LUBILOSA project (161) in West Africa and Oman. Extensive collections have also been undertaken in Madagascar (137) and Burkina Faso (140). In addition, with the publication of relevant books (97) and manuals (112) there has been an increased awareness of these pathogens, and further deposits to culture collections are made regularly.

Several methods are available for collecting insect pathogens; whereas isolation of pathogens from soils reveals many isolates, these need extensive screening to determine the most virulent. On the other hand, searching for diseased insects in the field yields few isolates, but most are virulent. The majority of isolates have been collected by caging random samples of insects and keeping them until they die; this is the most productive technique, and the majority of pathogens that are found are virulent (161). The theoretical basis for preferring the cage technique is that a small fraction (1%–4%) of the acridid population may be expected to harbor latent infections. In the field, these may recover or, if they become moribund, may be taken by predators or scavengers. In the cage crowding and stress induce apparent infections; any grasshoppers that behave abnormally or die can be removed and diagnosed.

The majority of virulent isolates found so far were *Metarhizium anisopliae* var. *acridum* Metsch. Sorr. (43), although isolates of other varieties of *M. anisopliae*, *Beauveria bassiana* (Bals.) Vuill. and *Sorosporella* sp. (158) were also found.

Natural Prevalence of *Metarhizium*

Although *Metarhizium* was the most common grasshopper pathogen found in West Africa, the prevalence levels were generally low. Continuous monitoring over 6 years at Malanville in the Niger flood plain in north Benin showed prevalence levels of 2%–6% (159, 160). Because some susceptible grasshopper species, particularly *Pyrgomorpha cognata* Krauss, were present throughout the dry season, it is possible that *M. anisopliae* survives the dry season by constantly cycling at a low level. However, observations (CJ Lomer & M Thomas, unpublished data) also indicate that spores can persist throughout the dry season in the cadavers of wet-season species, particularly in heavily sclerotized thoracic or femur segments (a cadaver of *K. angulifera* containing viable spores was found in north Benin towards the end of the dry season in April 1993). The ability of the pathogen to

survive through the dry season in infected cadavers and to initiate infections in the following year was subsequently demonstrated (175).

Similar low levels of prevalence of *Metarhizium* were observed in *Zonocerus variegatus* (L.) populations in southern Benin (141) and are consistent with the recovery rates of *Metarhizium* in cage collections of locusts in Madagascar [W Swearingen, Montana State University, unpublished data; see (137)].

Epizootics of *M. anisopliae* have been observed on a few rare occasions. An epizootic affecting *Ornithacris cavroisi* (Finot) was observed near Niamey, Niger, in 1989. Further epizootics have been observed affecting *H. daganensis* and *K. angulifera* at oviposition sites in Malanville and *Diabolocatantops axillaris* (Thunberg) in Chad, both in 1991 (117). The neotype of *M. anisopliae* is described from an epizootic affecting *S. gregaria* in Eritrea in 1960 (6, 185).

Theoretical considerations indicate that the most virulent pathogens will only be found at low levels in locust and grasshopper populations (59); the observed natural prevalence of *Metarhizium* fits this concept well. While our data are not adequate to construct a quantitative model, we can say that in areas where *Metarhizium* is present at enzootic levels, epizootics can occur under favorable (cloudy and rainy) weather conditions and high host population densities.

Bioassays

Bioassays can take the form of rapid screening or more complex assays that take into account actual field conditions and varying doses. Initial screening by LUBILOSA (9, 148) showed that some *Metarhizium* isolates have outstanding virulence compared with others, and that isolates from dead insects were generally more virulent than isolates from soil. *Metarhizium* isolates were consistently more virulent than isolates of *B. bassiana*. A dozen of the more virulent isolates were selected for further work. Ideally, all of the characteristics mentioned above would have been evaluated. In practice, one virulent isolate, IMI 330189, isolated from an epizootic affecting *O. cavroisi* near Niamey, Niger, was submitted for mammalian safety testing, and further development work focused on this isolate.

A fluctuating temperature bioassay, mimicking actual internal locust body conditions, was developed by Jenkins et al (78). Although other isolates reduced median lethal times by about 10%, compared with the standard IMI 330189, this slight performance premium was judged insufficient to warrant repeating the safety and other tests necessary to bring a new isolate to the same development stage as IMI 330189. This standard isolate was tested against a range of different Sahelian grasshoppers and other locusts (113). It was effective against all acridid species. The pyrgomorphid *Z. variegatus* (L.) was less susceptible, and a homologous isolate (from this species), I91-609, was found to be more virulent and was field-tested (41, 115) but not, in the end, commercialized. A similar result was observed for other isolates; in general, the most virulent isolate is that from the same species and from the same locality.

From careful examination of laboratory mortality curves, it is clear that some isolates may kill quickly at high doses, whereas at low doses, different isolates may cause higher mortality. A three-dimensional (time, dose, mortality) analysis technique was developed by Nowierski et al (137) and used to screen *Metarhizium* and *Beauveria* isolates from Madagascar.

Characterization

Methods for characterizing fungal isolates have evolved rapidly over the last decade. A suite of biochemical techniques was used by Bridge et al (22, 23), whereas Bidochka et al (15) used random amplification of polymorphic DNA. With increasing data available, a contradiction between the classical taxonomy of the genus *Metarhizium* (181) and biochemical data became apparent. The classical definition of the species within the genus is based on spore size and shape; isolates from acridids had variable spore shape, but were consistent in their biochemical characteristics. Thus biochemically close isolates would sometimes be classified as *M. anisopliae* and sometimes as *Metarhizium flavoviride*. This was formally resolved by Driver et al (43), who proposed the variety *acridum* in the species *M. anisopliae* based on the use of internally transcribed spacer ribosomal DNA (rDNA) sequence data. Publications on *M. anisopliae* var. *acridum* IMI 330189 before 1999 use the name *M. flavoviride*. It is worth noting that the telomorph (perfect, or sexual) stage of *M. anisopliae* is not yet known, and a further name change may be necessary in the future. The telomorph of *Metarhizium taiii* has been described by Liang et al (106) as *Cordyceps taiii*.

Although the *M. anisopliae* var. *acridum* cannot be distinguished from other *M. anisopliae* on the basis of spore size and shape, it has a number of distinct characteristics in addition to its internally transcribed spacer rDNA sequence data. It is cosmopolitan and has been found affecting grasshoppers or locusts in all tropical countries where consistent searches have been made. Its host range is restricted to Orthoptera: Acridoidea and it has the capacity for limited growth at 37°C. Biochemically, it has the capacity to metabolize α -fucosidase and has a distinctive catalase isoenzyme band (23).

These results are in accord with a general model for deuteromycete fungal isolate comparisons described by Maurer et al (125); both *Metarhizium* and *Beauveria* are generalist fungal genera, which sometimes specialize on insects. Both generalist and specialist strains can be isolated and characterized using molecular markers. Insects may be less or more resistant to fungi; generalist strains can be used to kill less-resistant insect species, whereas specialized fungal strains may be needed to kill resistant insects. For locusts and grasshoppers, the most effective specialist strains all belong to the *M. anisopliae* var. *acridum*. Other fungi can kill grasshoppers and locusts; as we discuss below, the insects' best defense is thermoregulation. Under conditions that do not allow these insects to thermoregulate, other more generalist isolates may well appear as virulent as *M. anisopliae* var. *acridum*; however, the *M. anisopliae* var. *acridum* isolates will be most

effective under field conditions. It is noteworthy that four independent programs, LUBILOSA, Australia, Madagascar, and Brazil, have all selected isolates of *M. anisopliae* var. *acridum* for development.

Features of the Biology of *Metarhizium*: Spore Quality, Storage

It is worth spending a few lines summarizing particular features of the fungus *Metarhizium*. As mentioned above, and in common with all deuteromycete fungi, the sexual stage of the fungus is not known, and the life cycle is simple. Conidia are the only type of spores produced; no sexual recombination is involved in the formation of these conidiospores. These spores germinate within ~16–20 h of being placed under favorable conditions of high humidity and suitable substrate. A single fungal hypha emerges from the conidium which, on sterile agar, grows to form a mycelial mat. On the other hand, on the surface of an insect the hypha develops a specialized structure known as the appressorium which attaches to the cuticle. This appressorium forms a peg which uses enzymes (proteases and chitinases) and mechanical pressure to penetrate the cuticle. Inside the insect, the mycelium continues to grow, but it buds to produce free-floating yeastlike cells called blastospores. The blastospores multiply in the haemocoel, in some cases producing toxins. Under conditions that are favorable to the fungus, *Metarhizium* is able to overcome insect defense reactions and kill the insect. The fungus then switches back to mycelial growth. As the insect cadaver dries out, the fungal mycelium penetrates the insect cuticle, particularly where it is thin, and conidia are then produced on the outside of the insect (89). A particular feature of *M. anisopliae* var. *acridum* is that, under dry conditions, the insect cadaver is hollowed out and conidia are produced inside the cuticle. This mechanism appears to protect the conidia from sunlight, and they may be released as the cadaver breaks up.

The process following application of biopesticide is similar to the natural infection process. In this respect, *Metarhizium* biopesticide differs markedly from biopesticides such as *Bacillus thuringiensis* that kill by means of a toxin. Dry conidia are naturally highly infectious and capable of adhering to the insect cuticle; in the case of a biopesticide, the formulating oil helps the spores to stick to the insect cuticle. This adhesion process, and the subsequent germination of spores, is dependent on cuticular fatty acids; attempts to improve formulations by manipulating fatty acid components were not successful (8). Other attempts were made to find ways of reducing the lag time between application of spores, germination, and penetration. Presoaking spores in water before application was tested, but this procedure was not found to be advantageous (132).

One of several technical challenges to the large-scale implementation of *M. anisopliae* use was the short shelf life of conidia produced under standard conditions. This problem was found to be caused by the presence of excess moisture in the spores (55, 131); furthermore, the spores are highly hygroscopic and under ambient atmospheric humidity can rehydrate rapidly. By extending viability models

originally developed for plant seeds, a storage model was developed and validated (60–62). Application of this model has extended the spore storage time from a few weeks to up to 4 years. Time-temperature indicators (Lifeline Company; 107) have been calibrated against the model and experimental spore storage results and are routinely attached to spore packages to indicate whether the spores have been exposed to temperatures high enough to reduce viability.

UV irradiation is a common cause of spore inactivation in the field. Considerable research effort has therefore been invested in finding ways to prolong spore survival in the face of UV radiation. Different formulation types have had some impact on spore survival (1). Several sunscreens were investigated under laboratory conditions (65, 129); these studies were extended to field conditions by Shah et al (157) but were not found to affect the efficacy of the fungus in the field. A more promising approach might be to exploit the natural variability in UV tolerance. Fargues et al (48) compared many *Metarhizium*, *Beauveria*, and *Paecilomyces* isolates. After 4-h exposure to simulated sunlight, Fargues et al observed a 35% survival for the best acridid isolate (the Australian FI985) compared with 1%–2% rate among the most susceptible isolates. IMI 330189 showed a survival rate of 7.5%. Despite these laboratory results, field data indicated survival of *Metarhizium* IMI 330189 spores for >1 week in north Benin (174) and >6 weeks in Niger (104).

Mass Production

In industrial terms, liquid fermentation of microbial control agents is more advanced than solid-state fermentation and has the potential for much lower cost production (74, 166). However, the conidia of the hyphomycete fungi are the only stage with good environmental persistence; the conidiospores of *Metarhizium* and *Beauveria* are hydrophobic and are only produced at an interface between substrate and air. Several companies worldwide have the capacity for solid-state fermentation, but none were willing or able to produce *M. anisopliae* var. *acridum* to LUBILOSA specifications in the early 1990s. A spore production pilot plant was therefore constructed at the International Institute of Tropical Agriculture (IITA) in Cotonou, Benin. The production method was adapted from similar plants in China and Brazil (126) and involved an initial liquid phase in sucrose/brewer's yeast broth, followed by a solid phase on sterilized rice in polypropylene bags (33, 75). Although the pilot plant was uneconomic to run on a large scale, it was an essential research tool without which neither optimization of fermentation process characteristics nor field testing would have been possible. For instance, a prolonged conditioning period of ~2 weeks during which the spores dry on the rice substrate was found to be essential to produce spores with good storage characteristics (131).

Spore extraction was another important step in developing a refined product. Normally, spores are separated from their substrate by simple mechanical sieving. However, for a product intended for ultra-low-volume (ULV) application, a uniform particle size is essential. In particular, even substrate particles the same

size as the spores must be excluded, because these particles may subsequently absorb moisture and swell. To achieve the uniform particle sizes needed for ULV, a cyclone air-stream device was developed, which is now available commercially.

The mass production of *B. bassiana* is well advanced (74) and, in particular, the production units of Mycotech Inc. in Butte, Montana, are able to produce spores in high volume at low cost (73, 135).

Investigations on production of *Metarhizium* in liquid fermentation are proceeding; both blastospores and aqueous conidia are produced in this way (76, 90). Stephan et al (166) describe a low-cost blastospore production system based on waste from poultry production and a method of freeze-drying the formulation.

An economic model for mass production was developed by Swanson (170), who concluded that the bag production system could not compete with large-scale solid-state fermentation systems. This finding has been supported by analyses of the IITA production plant (33); to provide a cost-effective product to clients, the LUBILOSA production system was transferred to the private sector.

Formulation and Application

The formulation and application of biopesticides has been a much-neglected area, but recent publication of a book on the subject corrects this gap (24). In the case of locust and grasshopper control, the majority of control operations are undertaken as ULV applications of oil formulations. Because control operations often take place in remote desert areas, the ability to avoid reliance on water supplies is important, as is the high work rate and convenience of ULV. Because early experiments had demonstrated the increased efficacy of oil formulations of fungal spores under bioassay conditions (10), the LUBILOSA project focused on the development of an oil formulation of *M. anisopliae* spores suitable for application by ULV. Two formulations were developed: a low-tech formulation suitable for hand-held or vehicle-mounted sprayers, and a high-tech formulation suitable for aerial application. The low-tech formulation consists of a mixture of either vegetable oil (70:30 kerosene:peanut oil) or mineral oil (50:50 Shellsol:Ondina); the details of the high-tech formulation are confidential and form part of the technology transfer package discussed below.

Oil formulations were tested in a range of commonly used spinning-disk sprayers, including the hand-held Micron Ulva-Plus, vehicle-mounted Ulva-Mast, and aerial Micronair 8000. Formulated spores were able to survive passage through the exhaust nozzle sprayer (52). An aqueous formulation was also developed (77).

Bait formulations of mycopesticides have been considered (130); for locust control in Africa, baits are impractical (21), but in other circumstances they could be effective (68, 83, 86). Application to oviposition sites has also been considered (69).

Metarhizium IMI 330189 Field Trials in Africa

The central problem of field trials of slow-acting control agents against migratory pests is always that the treated insects have the opportunity to disperse before the

control agent has a measurable field impact. Use of large treatment plots is the obvious solution, but in the early development stages of a biopesticide, only small quantities of active agent may be available and the development of alternative experimental formats becomes important.

Cage and Arena Trials Cage trials are useful but ULV application relies on a light wind to deposit droplets, whereas cage mesh generally impedes airflow. An application technique that uses a fan to provide the air stream (Micron Ulva-Fan) was developed and used in cage trials on desert locusts in Niamey. A simpler technique was found to be the so-called arena trial format, in which locusts are released into cleared arenas where either a food source or wingclips prevents flight; a standard ULV spinning-disk sprayer is then used to apply the mycopesticide formulation. Standard spray-monitoring devices, such as oil-sensitive paper, agar plates, or glass slides, can be posted at the arena. This trial format is very useful and versatile in testing formulations and application techniques and as a training tool (11).

Field Application with Cage Incubation An extension of the arena trial technique is to treat grasshopper or locust populations in the field and then to catch samples from the treated plot for cage incubation before the insects have an opportunity to disperse. Cage incubations provide clean data because the sample size can be carefully controlled and daily observations are possible; the drawback is that cage conditions do not necessarily reflect field incubation conditions. Generally, disease incubation is more rapid in cages than in the field, because insects cannot effectively thermoregulate in cages (see below). As with arena trials, cage incubation data have their place as a training tool and provide useful data under some circumstances. The first evidence for field infection in grasshoppers in Africa following mycopesticide application was obtained by this method using *M. anisopliae* var. *acridum* strain I91-609 against the variegated grasshopper in southern Benin (115). This trial was subsequently extended to provide the first demonstration of field population reduction on 1-hectare (ha) plots (41).

Green-Island Trial Format One further experimental format for the desert locust is worth discussing. This is the green-island in which patches of millet or maize are planted and irrigated in an otherwise dry area. The patch can be infested with locust nymphs, and treatment and observations can be made at any time up to maturation, normally \sim 10 days after the last molt. Although the green-island format worked moderately well as far as confining the locusts went, the green patch proved highly attractive to birds and lizards and few locust nymphs survived to maturity, irrespective of the treatment.

Field Trials in West Africa The rice grasshopper, *H. daganensis*, is relatively immobile, and plots of 4 ha were large enough to demonstrate a field impact for *Metarhizium* (118). Similar-sized plots were used to demonstrate an effect on

mixed young nymph populations in Mali (42). Older Senegalese grasshoppers and other Sahelian species are more mobile; first results were obtained on 50-ha plots in Niger (92). In a comparison with a toxic chemical standard, fenitrothion, in 1995, *Metarhizium* outperformed the chemical in every respect except speed of kill. Fenitrothion killed grasshoppers immediately, but after 10 days, insects reinvaded the plots. Meanwhile, in the *Metarhizium*-treated plots the counts were declining steadily, so that after 10 days, counts in fenitrothion and *Metarhizium* plots were more or less equal. After 10 days, the counts in the fenitrothion plots continued to recover, whereas counts in the *Metarhizium* plots continued to decline. Because the field season lasts only 2–3 months, a single application of mycotoxicide provided season-long control, whereas repeat applications of fenitrothion would have been necessary to achieve such control. This result was confirmed in 1998 trials with aerial application on 800-ha plots (104).

Further Field Trials in Africa *M. anisopliae* var. *acridum* IMI 330189 has also been tested and found to be effective against the brown locust *L. pardalina* (147) and the tree locust *Anacridium melanorhodon melanorhodon* (Walker) (91).

Extensive trials have been conducted against the desert locust. For this highly mobile insect, we estimate that a plot size >1000 ha would be necessary, and such trials are expected to take place within the FAO Emergency Prevention Scheme program. To monitor trials on smaller areas, Langewald et al followed treated locust bands in Mauritania (103).

Field Trials with Other *Metarhizium* Strains Outside the African Mainland

Australia In Australia, trials with *M. anisopliae* var. *acridum* FI984 followed a pattern similar to the trials in Africa. An indigenous isolate, FI 984 isolated from *Austracris guttulosa* was found to be pathogenic to target species in laboratory tests (128). In the beginning, working with the wingless grasshopper *Phaulacridium vittatum* (Sjöstedt), a less mobile species, field trials demonstrated population reductions (4, 127). When Australian plague locust populations became available, trials were conducted and were also successful against this species (63). Trials have now progressed to 50-ha plots and the FI 984 mycotoxicide has been registered.

Madagascar In Madagascar, the indigenous *M. anisopliae* var. *acridum* isolate SP9 gave promising results against *L. migratoria* in the laboratory (137) and on 10-ha plots (40); it has now been registered (121; D Swanson, personal communication, 2000).

Brazil In Brazil, the indigenous isolate CG423 was found to be virulent to the pest species *Rhammatocerus schistoceroides* Rhen (119) and it subsequently has been field tested (120), with encouraging results.

Beauveria bassiana

B. bassiana was tested as a grasshopper mycopesticide in a bait formulation in Canada (83), in a ULV application in Mali (84), and in aqueous and oil formulations in the United States (73). Results were promising, and a commercial product has been registered (Mycotrol; see 135), but subsequent trials under a range of weather conditions indicated that grasshoppers were frequently able to escape infection by thermoregulating above the permissive temperature for fungal growth (6, 73). However, as discussed above, *B. bassiana* production facilities are already in place, and isolates with higher-temperature tolerance are known (140). It is possible that more effective acridid mycopesticides based on *B. bassiana* could still be developed; mixtures of fungi are proposed by Inglis et al (66).

Ecological Studies

Spore Persistence and Recycling We can distinguish three routes by which grasshoppers may be infected by *Metarhizium* biopesticide application: direct impact by droplets, secondary pickup from vegetation and soil, and from spores produced on infected cadavers (referred to as horizontal transmission or recycling). Modeling studies have been conducted to examine which of these routes of infection is most important and to identify what relative effects they have when combined (179). Direct impact can be assessed by including a fluorescent tracer in biopesticide formulation and by counting droplets on hoppers under UV light (115). The importance of the other infection routes can be assessed by inference from the number of insects that die without visible droplets; however, the picture is complicated by insect molting (105).

It has been possible to demonstrate that *Metarhizium* can sporulate on cadavers in the field, cause infections, and survive between seasons (2, 175). Heavy predation and scavenging in the Sahelian environment eliminate >90% of infected grasshoppers (2). Although theoretical considerations point to the potential importance of density-dependent horizontal transmission (177, 178), it has not been possible in practice to distinguish prolonged spore survival from recycling of spores from cadavers (2, 104). A definitive experiment to determine the proportional contribution of each component would involve an initial application of a short-persistence pesticide to reduce the grasshopper population, followed by application of *Metarhizium* spores. The difference in persistence of spores between the with-grasshoppers and without-grasshoppers plots would indicate the contribution of spore recycling.

Thermoregulation and the Issue of Speed of Kill In field use, *Metarhizium* has a relatively slow and variable speed of kill, which may create practical problems for its use. Studies have shown that environmental temperature and host thermal biology are central to the interpretation of the patterns of mortality observed following spray applications because many grasshoppers and locusts actively thermoregulate to maintain their body temperatures around a preferred set point during the day

(31, 98, 187). They use orientation to the wind and sun, position on vegetation, and position in temperature gradients to control their internal temperature (98–100). This internal body temperature can be significantly different from ambient temperature and can be maintained for a number of hours, given the right environmental conditions. For example, the preferred body temperature of key species such as the Moroccan locust, desert locust, Senegalese grasshopper, and certain North American rangeland grasshoppers has been shown to be in the range 38°–40°C (18, 20, 67). This temperature represents the upper limit for growth of *M. anisopliae* var. *acridum* (48a, 176) and is above the upper limit for *B. bassiana* (67). Furthermore, some studies have indicated a behavioral fever response, in which locusts thermoregulate to a higher preferred temperature as a response to fungal infection (19, 25). Thus, under conditions that enable locusts and grasshoppers to thermoregulate optimally, these pathogens are unable to develop inside infected insects and incubation time will be increased accordingly. Speed of kill may also be restricted by low nighttime temperatures; temperatures of 5°–10°C are not unusual under desert conditions. This drop in temperature often results in a relatively small window for pathogen growth over a 24-hour period and a long survival time, especially if these cool nighttime conditions coincide with optimal thermoregulatory conditions during the day. Conversely, under cloudy conditions and ambient temperatures in the range 26°–32°C, effective thermoregulation may be impossible, leading to rapid fungal growth and reduced incubation time.

It is clear that an understanding of the thermal ecology of the host-pathogen interaction is central to determining the likely performance of the mycoinsecticide. GIS has been used to combine spatial and temporal variables with insect growth models (88) and grasshopper outbreaks in Canada (81). We intend to develop a similar system for Africa; the aim is to develop maps that describe likely pathogen performance across the season at different locations.

Environmental Impact and Registration

Biopesticide Registration Requirements The data required for registration of biological pesticides are in a state of flux worldwide. Recent stabilization of the US registration guidelines (182) has done much to encourage a bolder and more positive approach to biopesticide registration. In most developing countries, specific biopesticide registration guidelines are not available, and biopesticide registration applications are treated on a case-by-case basis under chemical pesticide guidelines. Registration normally applies to a single defined isolate, for which mammalian toxicity data is necessary; often, published data for closely related isolates can be accepted at the discretion of the registration authorities.

Mammalian safety testing to US Environmental Protection Agency standards (164) have indicated that *M. anisopliae* var. *acridum* IMI 330189 has no impact on humans or other mammals in the field.

Further registration tests included the impact of *Metarhizium* on honeybees, parasitic Hymenoptera, and a variety of biological control agents in culture at IITA

(113). Reports indicated that *M. anisopliae* var. *acridum* had a negative impact on parasitic hymenoptera under laboratory conditions (39). However, this effect was not confirmed under cage (167) or field (142) conditions.

Previous registration of different *M. anisopliae* isolates in Europe and the United States made registration of *M. anisopliae* var. *acridum* simpler. Published data include tests on bees (7), mites, and weevils (113). Tests on mammalian toxicity and aquatic crustaceans form part of the confidential registration package. Provisional registration has been granted for *M. anisopliae* var. *acridum* IMI 330189 in South Africa and registration is pending with the Sahelian authorities. The FAO desert locust pesticide referee committee has also recommended *M. anisopliae* var. *acridum* IMI 330189 for use in environmentally sensitive areas (47). Similarly, in Australia, *M. anisopliae* var. *acridum* FI 985 has been registered for organic farming, meaning that it is currently the only control agent available for locust control in organic beef production areas. In Madagascar, *M. anisopliae* var. *acridum* SP9 has also been registered (121).

Further Environmental Impact Tests Registration guidelines tend to be developed for temperate ecozones and risk, ignoring some important tropical ecosystem components, in particular lizards. Previous work by the FAO locust insecticide toxicology project, LOCUSTOX, in Senegal showed that lizards are frequently affected by chemical pesticides; *Metarhizium*, by contrast, showed no adverse effects (R Peveling, Basel University, personal communication, 1999). The impact on birds was tested by feeding sick grasshoppers to test birds in experiments in Canada; no effect of the fungus was found on growth, survival, weight gain, behavior, or histopathology of the birds, based on sectioning and examination of eight organs (165). Further work is planned on the impact of *M. anisopliae* var. *acridum* on nontarget grasshoppers. Thus far, it would be reasonable to conclude from the data available to date that use of *M. anisopliae* var. *acridum* is without environmental risk.

Comparisons with Chemical Pesticides In addition to indicating a lack of risk associated with the use of *Metarhizium*, it is important to establish that *Metarhizium* in use actually offers environmental benefits over chemical pesticides. One of the principal constraints that affect environmental impact studies in the field is the huge number of samples that are usually collected. In creating test formats to compare the impact of *Metarhizium* against chemical pesticides, Peveling et al (142) developed a simplified presence/absence test procedure, which permitted the processing of data within the time between trials. A range of key indicator species was identified, and traps were designed to monitor these species. Traps were deployed in the field only for a predetermined time slot. Using this technique, Peveling et al (142) compared *M. anisopliae* var. *acridum* with standard chemicals in operational-scale field trials against the Senegalese grasshopper. Whereas *Metarhizium* had no impact on any nontarget organisms, several orders of inoffensive insects were affected by fenitrothion.

Operating Characteristics of *Metarhizium* Biopesticides

From the point of view of the locust-control officer, *Metarhizium* biopesticide is received as an oil-flowable concentrate, not unlike a chemical pesticide, although it needs to be protected from excessive heat and direct sunlight. Containers bear a time-temperature indicator, which indicates whether the container has been exposed to temperatures likely to prove injurious to the spores. Once formulated, the spores stay in suspension without need for agitation, and the operator can apply *Metarhizium* biopesticide in the same way as any chemical pesticide. Normally, once diluted for application, the formulation is used within several days. If applied correctly, the first insects should be observed dying in the field within 10 days; a sample of live insects collected 1 day after spraying and kept under shady conditions should show signs of mortality after 7 days. The full effect of between 70% and 90% mortality should be seen after 14–20 days, with shorter times under overcast conditions, and longer times under hot, sunny conditions. Normally, the majority of sick and dying insects will be taken by predators and scavengers, and mycosing cadavers will rarely be seen although a few, with the characteristic red color, may be found (116).

Most trials on *Metarhizium* have been conducted at a dose rate of 100 g or 5×10^{12} spores ha^{-1} . Although the final cost of the product is not yet known, indications are that at this application rate, the product cost would be higher than equivalent chemical pesticides. Trials at lower dose rates indicated little loss of efficacy at 50 g ha^{-1} in Mali (OK Douro-Kpindou, IITA, personal communication, 1998), and in Australia the rate was decreased to 10 g ha^{-1} , with slower but still acceptable results (G. Hamilton, APLC, personal communication, 1999).

Technology Transfer and Implementation

Biological pesticides have many favorable properties in IPM terms but are seldom profitable to the producer; apart from the bacterium *B. thuringiensis*, there are few fully commercial success stories (151). A broader definition of success leads to a more optimistic picture and shows how public sector agencies can lower the entry barriers to commercial involvement (49). In the case of LUBILOSA, the particular feature is the heavy research investment by the multiple public agencies involved, and the transfer of this technology to private-sector production companies. This technology transfer process involved (a) definition of the product, definition of the parties to the agreement, and an inventory of the intellectual and germplasm property involved; (b) a valuation of the property and investments by the parties; (c) licensing agreements to two production companies; and (d) design of a trust fund to absorb license and royalty payments. In essence, the donors and other contributors agreed to transfer their intellectual property rights to LUBILOSA. Although the vast majority of the project data was published, certain key details and technical know-how was reserved for the companies. CAB International, acting on behalf of the project and its partners, licensed two commercial companies to produce *M. anisopliae* var. *acridum* IMI 330189 to carefully defined

specifications under the trade name Green Muscle®. The specifications include definitions of spore powder concentration, strain identification, virulence, moisture content, and level of contaminating microbes. Each company was allocated a certain geographical region of operation. The license fee and any royalty payments are accumulated in the LUBILOSA trust fund; disbursements from the trust fund are made to developing countries pursuing the development of biological pesticides.

Near the start of the project, a protective patent (GB 2255018B) on oil formulation was taken out on behalf of the project by CABI Biosciences with the objective of protecting the right to continue the research in case patents were taken out by other organizations. The international agreement on the Convention on Biological Diversity in 1993 clarified the issue of germplasm ownership and the involvement of the African national programs; the *M. anisopliae* var. *acridum* strain IMI 330189 originates in Niger, and the Nigerien authorities were involved in the development of the technology transfer agreements and will be among the primary beneficiaries of trust-fund disbursements. The Convention does not give quantitative guidance on the weight to be attached to original germplasm ownership compared with intellectual property rights input and development; but involvement of the germplasm owner in subsequent development is considered to be of greater value than reimbursements of small royalty payments (70).

OTHER MICROBIAL CONTROL AGENTS

Metarhizium has emerged as the first-choice inundative microbial control agent but it has little intergenerational persistence. We now consider microbial control agents that might be expected to function as inoculative or classical biological control agents.

Microsporidia

N. locustae was the first microbial agent to be developed as a biopesticide for locust and grasshopper control (56, 58; see 82 for a review). It is a spore-forming protist, which initiates infection in grasshopper midgut and subsequently spreads to the fat body. Because *N. locustae* must be ingested to infect acridids, spores are usually applied on wheat bran carrier (bait). Typical *N. locustae* bait application equipment includes hand-held cyclone seeders, truck-mounted bran spreaders, fixed-wing aircraft of various sizes, and helicopter-mounted applicators. Although infection may occasionally result in high levels of mortality among some acridid species (169), realistic goals of application of this pathogen include reduced feeding by infected insects (87, 139), delayed development (155), and lower reproduction (109). *N. locustae* spores are produced *in vivo* in *Melanoplus differentialis* (Thomas) (57).

N. locustae has been extensively field-tested in the United States and Canada, and releases were also conducted in Argentina, Cape Verde, China, and Mali.

Nosema was registered and produced by Evans Biocontrol of Montana and is available from various organic-farming outlets. Comprehensive studies of the impact of *N. locustae* applications have been conducted in Argentina and indicate a considerable decline in locust populations in the treated area, as well as a spread in pathogen incidence (102). In China, areas of 100,000 ha have been treated annually, to the apparent satisfaction of the authorities (191). Follow-up evaluations of the releases in Mali and Cape Verde are lacking. Thus, for *N. locustae* we see a situation where a narrow evaluation of a control agent in terms of its immediate impact fails to assess its full benefit. Perhaps, combined with *Metarhizium* in an IPM context, its full benefits can be realized.

Several other microsporidians with virulence far greater than that of *N. locustae* have been described, including *Nosema entomophaga*, *Nosema cuneatum* (82), and *Johenrea locustae* (101). Because of their greater virulence, developing a production system may be difficult, but if these agents are to be used as classical or inoculative biological control agents, high production costs may be acceptable.

Entomophthoralean Fungi

The entomophthoralean fungi are a group of fungi with complete and complex life cycles. They are of interest as classical biological control agents because of their observed capacity to cause spectacular epizootics (30, 53). The species affecting grasshoppers, *Entomophaga grylli* (Fresenius) Batko, has long attracted attention as a potential biocontrol agent and was briefly marketed in South Africa in 1898 as a biopesticide (145). Detailed studies on epizootiology have been carried out in United States (27) and Benin (141), but artificial culture has never been successfully developed. The difference between biotypes of *E. grylli* was explored by Bidochka et al (16) and several pathotypes were described. In particular, pathotype III, also referred to as *E. praxibulli*, from Australia, was found to have a wider host range and greater virulence than indigenous North American pathotypes, and was accordingly introduced as a classical biological control agent (27). Initial results were encouraging, but incidence declined after a few years (17). Nevertheless, in other cases, entomophthoralean fungi have declined to low levels but given rise to massive epizootics many years after the initial release (e.g. *Entomophaga maimaga* in Gypsy moth, northeastern United States; 53).

ARTHROPOD AND VERTEBRATE NATURAL ENEMIES

Consideration of the population dynamics of grasshoppers leads us to focus on two biological control options using nonmicrobial agents. First, reduction in the use of chemical pesticides should enable naturally occurring, indigenous natural enemies, including birds, to have a greater impact. Second, the apparent discrepancy between the impact of the Australian egg parasitoids and those of Africa or North America leads us to consider the possibility of using the Australian *Scelio parvicornis* Dodd as a neoclassical biocontrol agent.

Egg Parasitoids

Grasshoppers and locusts lay their eggs in egg pods; these are buried in the soil in more- or-less well-defined localities. Because each female may lay only between one and four pods, it is often suggested that locating, digging up, and destroying egg pods might be a viable control option (172). Some attempts have been successful, but in the absence of incentives we find that farmers seldom prioritize egg pod destruction over other agricultural, economic, and social activities. Various natural enemies attack locust egg pods (51); meloid beetles and the larvae of bombylid flies are effective predators, and hymenopterous parasitoids are also important. Because of their long historical use as successful biological control agents, the hymenopterous egg parasitoids offer the best prospects. The most important genus is *Scelio*, revised by Nixon in 1958 (136). *Scelio pembertoni* Timberlake was successfully imported to Hawaii as a biological control agent against the grasshopper *Oxya chinensis* (Thunberg) (34a, as cited in 51). Whereas the highest parasitism rates recorded are <10% in Africa and North America (110, 133, 158), Baker et al (3) recorded 30% for *S. parvicornis* in Australia.

The *Scelio* egg parasitoids are stenophagous rather than monophagous, and tend to select host egg pods according to habitat rather than species. Australian *S. parvicornis* was evaluated as a classical biological control agent for use in the United States. However, after acrimonious public discussion of the possible impact on native, nontarget grasshoppers, it eventually was not released (26, 108). For use in western Africa, the issues involved would be less complex because no grasshopper species are considered to be beneficial or endangered.

CONCLUSIONS AND PERSPECTIVES

To date, steps toward IPM of grasshoppers and locusts have been restricted to improving the accuracy of monitoring and forecasting and ensuring a rapid and effective response with the appropriate chemical pesticide. Despite advances in remote sensing, civilian remote-sensing equipment is not able to detect locusts, and its utility is restricted to defining areas where rain is most likely to have fallen, thus guiding the ground survey teams. With improved communications, use of Ground Positioning Systems and GIS, the collation and predictive value of the information collected is likely to be enhanced. There would be great benefits from further input in training and the application of modern information technology to locust population monitoring.

Newer chemicals, including fipronil and insect growth regulators, extend the portfolio of safer pesticides available to operators but are not without environmental risks, particularly in areas designated as environmentally sensitive. With the current availability of reliable mycopesticides based on *M. anisopliae* in Australia, Madagascar, west Africa, and South Africa, the possibility of building up a biologically based IPM scheme exists (114). *Metarhizium* has no measurable impact

on nontarget organisms and should enhance the impact of natural enemies, leading to the development of truly integrated pest management (173).

The mycopesticide is likely to be scarce for several years until production is scaled up to meet demand, and we would expect use to be focused on environmentally sensitive areas. Because of this limitation, and because its use against swarms is unlikely, the demand for mycopesticide may well be much more uniform than the demand for chemical pesticides. As with chemical pesticides, accurate forecasting and monitoring will remain essential. The mycopesticide is a living biological agent, and training in storage, application, and monitoring will be necessary. Further development of GIS based on climate variables will be necessary to ensure that the mycopesticide is used to its best effect and not under conditions that are unfavorable to its efficacy. These considerations are essential to gaining the confidence of locust-control officers, who will undoubtedly find *Metarhizium* biopesticide a change from their normal pesticides. Further technical improvements in *Metarhizium* biopesticides beyond the current Green Muscle® specification are likely to be modest.

We would not realistically expect to see >10%–30% of the current usage of chemicals replaced with biological agents over the next 5–10 years. However, at these levels, we hope to be able to determine whether current estimates of the enhanced role of natural enemies in pesticide-free environments turn out to be accurate. If so, we can expect to see a reduction in locust and grasshopper insecticide treatments that goes beyond the area treated with biopesticides.

Further followup work on *N. locustae* is essential. This exotic microbial agent has been released in Argentina and China with satisfactory results, but releases in Cape Verde and Mali have not been monitored long term. Research on other microsporidia is also important.

Evaluation of the dossiers prepared for release of *S. parvicornis* and *E. grylli* Path III in the United States should be conducted by the relevant African authorities with a view to the introduction of these agents to Africa, and possibly to other parts of the world.

Finally, donors and decision makers need to be aware that relatively modest expenditures on biological control, monitoring, surveillance, and prediction in a preventive control context will bring about considerable savings compared with the cost of reactively treating plagues that have escaped early vigilance.

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