Advances in Mycopesticide Formulation and Application

Chris Prior, Programme Leader, IIBC/IITA/DFPV Locust Biocontrol Research Programme, IIBC, Silwood Park, Ascot, UK

Introduction

The use of chemical pesticides has increased continuously since their invention in the 1920s and 1930s. They have enabled farmers to achieve high standards of pest control and thereby to produce high yields of blemish-free crops. Unfortunately, there are several well-known disadvantages to intensive use of chemical pesticides including environmental pollution, destruction of non-target organisms, induction of secondary pest problems and pesticide resistance. In order to achieve pest control without these problems, chemical pesticide use must be reduced and this has led to the concept of integrated pest management, where non-chemical control methods - biological control, cultural control and host plant resistance are given greater prominence in management systems.

Biological control is increasingly an important component of pest management systems and, in its most spectacular form may be the only management tool required. This is particularly true in the "classical" programmes of biological control where the pest is an imported species which multiplies in the new environment without its co-evolved natural enemies to control it. In such cases, the introduction of one or more of these natural enemies to the outbreak area may achieve spectacular control; the suppression of the exotic cassava mealy bug *Phenacoccus manihoti* in Africa by introduction of the parasitoid *Epidinocarsis lopezi* from the mealy bug's native home in South America is a recent example.

In other cases, however, control may not be achieved so easily. This is particularly true in the case of pests which are a problem within their native range, where introduction of co-evolved natural enemies is not possible. Even in the case of some exotic pests, the introduction of natural enemies may not lead to adequate control, possibly because these natural enemies do not flourish in the new environment or possibly because their impact on the pest is simply insufficient. In these cases, if biological control is to be used, then some form of augmentative use of natural enemies will be required. This may be done by the mass release of parasitoids, as in the case of *Trichogramma* spp, or it may be done by the use of "biopesticides". These latter are naturally-occurring pathogens of the pest, applied for control in the same way that a chemical pesticide would be used. There are many pathogenic micro-organisms that may be exploited for this purpose but only fungal pathogens will be considered here.

Fungal pathogens of arthropods

Arthropods, including most major agricultural, medical and veterinary pests, are attacked by a wide range of microbial pathogens including viruses, bacteria, protozoa and fungi. There are numerous fungal genera that have no counterparts among the pathogens of vertebrates and these entomopathogenic fungi are of particular interest for five reasons:

1. They are very diverse, attacking all major pest groups.
2. They penetrate the host directly through the cuticle and therefore do not need to be ingested to be infective.
3. They show a wide range of specificity and so may be exploited for control of both narrow and wide ranges of target pests.
4. Some can be grown easily on simple industrial substrates, making them suitable for mass production.
5. They have been extensively safety tested, in most cases without any adverse effects on vertebrates.

Because of their widespread occurrence, great diversity and sometimes spectacular ability to cause epidemics in pest populations, the entomopatho-genic fungi have long been claimed as potentially valuable pest control agents. However, attempts to exploit them for this purpose, which began in the last century, have seldom been successful. Two of the earliest of these attempts were in the Caribbean region: the "friendly fungi" in Florida and the control of sugarcane froghopper in Trinidad. The work is well summarised in Allard (1987) and Samson et al. (1988).

The "friendly fungi" are members of the genera *Aschersonia*, *Fusarium* and *Aegerita* which attack diaspidid and aleyrodid pests of citrus in Florida. In the early years of this century there was considerable interest in these fungi for pest control, based on the observation that natural epidemics could destroy insect populations in the field, and naturally infected material was sold to commercial growers to induce epidemics in their fields. The attempts ended in controversy because it could not be shown that these efforts led to improved control (Samson et al., 1988).

The sugarcane froghopper *Aeneolamia varia saccharina* is a major pest in Trinidad, originating from native grasses. In cane it multiplies rapidly, but suffers regular epidemics of *Metarhizium anisopliae* which may cause local collapse of the pest population, though unfortunately this usually happens after serious economic damage has been done. The pioneer entomologist and entomopathologist Rorer set up large-scale production of this fungus in Trinidad in 1910 and applied it to the cane in an attempt to control the pest, but again, a successful population reduction was never demonstrated (Allard, 1987).

After these and other attempts at using these fungi for control, interest lapsed for fifty years with the introduction of chemical insecticides. More recently, as problems with pesticide use have increased, there have been several large scale programmes to develop fungal pathogens for pest control. These include spraying *Metarhizium anisopliae* for sugarcane pests in Brazil (Mendonca,1992) and rice pests in the Philippines, spraying and soil granule application of *Beauveria brongniartii* for cockchafers in Switzerland (Keller, 1992) and spraying *Beauveria bassiana* for Colorado beetle in eastern Europe and USA, and corn borer in China. Among these programmes, the Brazilian programme continues and is often counted a success, although population reductions are not great compared to what would be expected from chemical spraying; the Swiss programme continues and a product is commercially available for soil treatment, but the aerial spraying programme has lapsed; the east European and Chinese programmes have lapsed; the Philippines work has not led to any implementation programmes. In fact, the record is not particularly encouraging and one may ask, why not?

Limitations on the effectiveness of fungal pathogens for pest control

Fungal pathogens in the genera most commonly investigated for pest control have no active means of dispersal and rely either on chance contact with the host, or on wind or rain to carry their spores to the host. This means that simply liberating the fungus in the pest's environment, as was done with the "friendly fungi", cannot achieve useful infection. This limitation has been largely recognised, and circumvented, by the use of spraying machinery to apply the fungi as sprays. However, a closer examination of spraying systems points to some possible limitations when they are used for applying fungal spores.

In order for a fungal pathogen to be an effective pest control agent, it must infect the host successfully. A farmer with a pest control product, the active ingredient of which is live fungal spores, must therefore suspend the spores in a suitable liquid (or dust them directly, but this is never done because of the difficulties in handling dusts effectively and safely) and atomise the suspension into small drops using a sprayer. These droplets must then either hit the insect directly, adhere to it and thus place the spore in a position where it can infect, or adhere to the foliage and be collected on the insect's cuticle later by secondary contamination.

Hazards for the spores occur at all stages. The spores of *Metarhizium* and *Beauveria*, the two most widely used genera, are dry, dusty and hydrophobic. They do not suspend easily in water and so are not easy to spray. In addition, there are a number of inherent problems associated with the spraying machinery used to atomise the suspension. The droplets produced by hydraulic knapsack sprayers or motorised mist blowers - the machines most frequently used for pest control in the tropics - are much larger than the optimum size for impaction on insects (Matthews, 1992). Thus the insect is bombarded with a relatively small number of droplets which contain many times the dose of spores needed to infect it. This is wasteful, since spraying is always inefficient and most drops miss the target; efficiency would be greatly increased if the suspension were broken up into much more numerous, optimally sized droplets. Those few droplets from conventional sprayers that do hit the target are likely to bounce off because insect cuticles are hydrophobic. Wetting and sticking agents can be used for chemical insecticides but many of these stickers cannot be used with spores because they are toxic. Where infection is achieved by spraying fungal spores, it is much more likely to be by secondary contamination of the insect from spores deposited on the plant surface than by direct contact, but here there are many hazards such as ultra-violet light and desiccation that reduce spore viability. These limitations reflect the fact that pesticide spraying is an imperfect process and much of the success of chemical pesticides is due to their very high activity rather than the efficiency with which they are applied to the target. It is notable that by far the most efficient transfer of pesticide to an insect target was achieved during aerial spraying of a locust swarm in flight, where only 5.5 per cent of the pesticide was transferred to the pest (MacCuaig and Watts, 1963); in other cases, the amount is invariably much less than 1 per cent.

In order to overcome these problems, application technologists have developed the concept of controlled droplet application (CDA). Recognising that there is an optimum droplet size for impaction on insects and that larger droplets are wasteful, CDA technology seeks to atomise all the pesticide into droplets of the optimum size, usually defined as about 70 µm diameter.

Under normal climatic conditions, water cannot be used to produce such small droplets, because evaporation is almost instantaneous and the droplets therefore become too small to impact and drift away. To overcome this problem, chemical pesticides are suspended in non-volatile liquid diluents. Oils are very suitable for this purpose.

A major advantage of CDA is that the volumes of spray that are needed can be greatly reduced when such small droplets are used, because all the pesticide goes into droplets of the optimum size and none is wasted. Further economies in volume are obtained by increasing the concentration of active ingredient and in some cases, pure active ingredient may be sprayed. Thus pest control can be achieved at application rates of <5.l /ha. Such low rates are referred to as Ultra Low Volume (ULV) application. The question is, therefore, can the advantages of CDA at ULV rates be extended to the use of fungal pathogens? It was with the objective of demonstrating the application of fungal spores by this technology that the IIBC/IITA/DFPV locust biocontrol programme was set up.

The IIBC/IITA/DFPV Locust Biocontrol Programme

Locusts and grasshoppers

Grasshoppers which can congregate to form very large swarms are called locusts and they cause enormous damage to world crops (Haskell, 1992). The most notorious is the Desert Locust, *Schistocerca gregaria*, which caused the biblical plagues and broke out across Africa and Asia as recently as 1986-1988. Some adults from this plague crossed the Atlantic and caused consternation by landing on some of the east Caribbean islands, although they did not cause any serious subsequent problems.

The adults of some locust species can fly hundreds of miles, which means that huge numbers of insects can suddenly invade agricultural areas. A large swarm of *S. gregaria*, for example, can weigh as much as 20,000 tonnes and contain ten thousand million insects, all of which can eat their own weight of green vegetation every day. Non-congregating grasshoppers are also very serious agricultural pests in many countries, and in the Sahel region of West Africa more than 20 species can damage crops. Control measures are often needed.

Chemical control of locusts and grasshoppers

Chemical pesticides have provided the only effective control method for locusts. The most effective of these have been the persistent organochlorine pesticides, especially dieldrin. However, the use of dieldrin was banned in the 1970s because of concern about its effects on the environment and in the last plague in 1986-1988, only less persistent pesticides such as fenitrothion were permitted. Very large amounts of pesticide were applied, often by air, but much of it failed to hit its target (Symmons, 1992).

This heavy and often ineffective use of pesticides has caused great controversy and concern, both in the afflicted countries and among the international donors who provide major financial support for control campaigns. As a result, there has been great interest in alternative, safer methods of control and one of these is biological control.

Prospects for biological control

Among the wide range of natural enemies of locusts and grasshoppers (Prior and Greathead, 1989), only the entomopathogenic fungi are suitable for development as biological control agents, for three reasons:

- "Classical" biocontrol using introduced parasitoids is not an option because the pest is native throughout its range. Augmentation of parasitoids is impractical because of the scale of the operation that would be necessary.
- Only those agents which can be mass-reared cheaply and quickly can be considered and this means that only a few microbial pathogens may be considered.
- Direct contact action is preferable because biological agents do not persist well in the desert conditions where control is carried out. The fungi are the only microbial pathogens which infect on contact.

Fungal pathogens as mycopesticides for locust control

Fungi exist which are unique, specific and highly virulent pathogens of insects and those in the genera *Metarhizium* and *Beauveria* fulfil the criteria we have described for a locust biological control agent. They have been tested in aqueous formulations against a wide range of pests, and in some cases have been used on a commercial scale. However, if they are to be used against locusts they must be adapted for use using Ultra Low Volume (ULV) technology (Lomer and Prior, 1992).

ULV technology was invented for locust control and is the only feasible way to spray locusts from the air, which is essential because of the large areas and remote location of much locust control.

When we began our research, we already knew that the dry, hydrophobic conidia of *Beauveria* could be suspended in vegetable oil and we also knew that this gave another, very great benefit, which is a large increase in the infectivity of the conidia (Prior et al., 1988). We believe this occurs because the oil causes the conidia to adhere more effectively to the insect, which has a waxy surface. During the course of the programme we have confirmed experimentally that formulation of *Metarhizium flavoviride* conidia in oil greatly reduces the LD_{50} (dose that kills 50 per cent of the insects in 5 days) for Desert Locust, even at the low relative humidity of 35 per cent (Bateman et al., 1993).

The central objective of our research programme is therefore to formulate these water-repelling conidia in oils suitable for ULV application and show that they will be infective under desert conditions. We thus aim to use existing and proven technology, but to replace the chemical pesticides with a biological alternative which has no adverse effect on the environment. Although we already knew that fungal conidia could be suspended in oils and would infect insects, we still had to show that oil suspensions could be sprayed effectively at appropriate rates using the existing machines, and we had to show that these suspensions would be infective under the hot, dry conditions where control operations are carried out. The results of our research on these topics have been very encouraging.

Research progress to October 1992

1. Selection of fungi

So far, we have examined over 100 isolates of *Metarhizium* and *Beauveria*, concentrating on those from Orthoptera. Only a minority have shown high virulence to Desert Locust. However, we now have virulent isolates from both within and outside Africa. In particular, we have more than 20 isolates of *Metarhizium flavoviride* from west and east Africa, all from acridoids and with high virulence to a range of acridoids including Desert Locust. We have chosen one of these, code IMI 330189 from Niger, as a standard for further development.

2. Infection at low humidity

In our assay experiments, the insects are inoculated with an oil suspension of conidia and held under standard conditions of a low relative humidity of 35 per cent and high temperature of 30°C. The five-day LD_{50} for conidia in oil is 8900 conidia/insect.

In contrast, water formulations will not kill 50 per cent of the insects at any dose in five days. Conidia formulated in water do in fact kill under the bioassay conditions of low relative humidity, but take about one day longer. This is an encouraging result: oil suspensions kill independently of relative humidity and more quickly than water suspensions (Bateman et al., 1993).

Oils do not affect viability of the fungus. Conidia of *Metarhizium flavoviride* in oil germinate on the locust cuticle after application in an oil suspension and form appressoria (the structure that the germinating conidium uses to penetrate through the cuticle of the insect).

3. Effects of ultra-violet radiation and high temperature

As well as low humidity, another hazard for conidia in the field is ultra-violet (UV) light, which can kill them very rapidly. We know from chemical insecticide work that locusts continue to collect insecticide by secondary uptake from deposits on the vegetation for several hours after spraying. It is therefore quite likely that there would be advantages in prolonging the residual life of the conidia for a few hours after spraying and this has led us to study UV protectants, or sun screens, and temperature tolerance.

The formulating oils themselves protect conidia from UV, but we can greatly increase survival by adding protectants. In the case of one of these compounds, 1 per cent oxybenzone which is widely used in suntan lotions, germination after three hours' exposure to artificial sunlight was increased from about 30 per cent to >80 per cent.

Another important question is, how long can the formulation survive at the high temperatures which it may encounter in the field? We have carried out experiments on storage of conidia of our standard isolate of *Metarhizium flavoviride* in various oils at different temperatures. When the conidia are dried correctly, they show no loss of viability after one year at 17°C in several oils suitable for formulation. Short exposures for a few hours to temperatures as high as 55°C do not reduce viability. It appears that the conidia are more tolerant of high temperatures than we had thought and we are working now to find the limits of this tolerance.

4. Field trials

We have carried out a preliminary field trial against *Schistocerca gregaria* at Niamey and Agadez, Niger with the kind assistance of GTZ. Adult locusts were collected in the field from their breeding area in Wadi Anu Makaren, north of Agadez, and sprayed there in the early morning using our formulation. They were returned to cages at Agadez for incubation. These results clearly show that the formulation can be taken to the field and sprayed effectively (Bateman, 1992).

We have also sprayed adult locusts in large wire mesh cages in the field at DFPV, Niamey. Temperature and humidity in these cages do not differ significantly from values in the field outside and mortality in these cages is therefore a demonstration of effective kill under field conditions. Adult *S. gregaria* sprayed in these cages with realistic field doses die in 6 - 12 days, with peak mortality on days 7 - 9.

Grasshoppers are also susceptible in the field. We sprayed field populations of the variegated grasshopper *Zonocerus variegatus* in the Lama teak forest in southern Benin. At the time of the trial the humidity was only 35 per cent. In samples taken directly after spraying, more than 90 per cent of the insects died of fungal infection. Similar results have been obtained with grasshoppers in field trials of up to 1 ha in Mali, northern Benin and Niger.

Conclusions

We conclude that:

There are isolates of *Metarhizium* with high virulence to locusts and grasshoppers.

We can formulate their conidia in oils for spraying at ULV rates using existing machinery. The formulations are more infective than water formulations and kill locusts at low relative humidities under both laboratory and field conditions.

First field trials in west Africa have shown that the formulations can be sprayed successfully against locusts and grasshoppers.

The major aim for the next phase of our programme from 1993 - 1995 will be to consolidate the field trial results in different environments against different grasshoppers and locusts. We will include some comparisons with formulations of a USA isolate of *Beauveria bassiana* which has proven very effective against grasshoppers in the USA and Cape Verde. The field trials will be carried out in close co-operation with Montana State University, GTZ and the national research programmes of Benin, Niger and Mali.

How can these ideas be extended to the control of other pests?

In principle, the advantages of CDA technology at ULV rates which have been demonstrated in our work on locusts and grasshoppers should be applicable to a wide range of pests. There is no doubt that suitably virulent isolates of *Metarhizium* or *Beauveria* can be found for most pests; the list of hosts already known for these two genera encompasses almost every insect order. However, we cannot assume that all pests will succumb to such sprays until some important questions are answered. To conclude, I will discuss briefly some of the questions that must be addressed if we are to consider using ULV formulations of mycopesticides against other pests.

Will this technology work on small pests?

Locusts and grasshoppers are large, often gregarious insects. They are excellent spraying targets, being noncryptic and easy to hit. Other pests are usually much smaller and may be well hidden in vegetation. They may be much harder to hit with CDA droplets. For example, whiteflies are usually on the underside of leaves where droplets seldom impact, and thrips are often hidden inside flowers.

Will secondary uptake be effective?

This leads directly from the above question: if the insects cannot be hit directly, they may still become infected from formulation which has impacted on the foliage. In this case, the effectiveness of the application will depend on how well the foliage is covered, how long the fungus survives and how readily it can be transferred from foliage to insect. There are many unknown factors at work here: we know little of the adhesiveness of formulations to leaves or of the effect of plant surfaces on spore survival.

Is spray drift a problem?

ULV technology often relies on drift to carry the spray to the target. This is not always desirable, because the wind may not be reliable and accurate placement of the spray may be impossible for farmers spraying small plots. The directionality and penetration of the droplet cloud can be enhanced by air-assisted spraying, where the rotary atomiser is mounted in the air blast from a mist blower so that the farmer can direct the droplets. This technology adds to the cost of application, however.

Drift of chemical pesticides may be undesirable because of the problem of contamination of non-target areas (exodrift). However, since biopesticides are inherently safer than chemicals, this should not be a problem. In any event, the greatest problems of drift arise with the very small droplets of <20 µm produced by conventional sprayers. Good CDA minimises the production of these very small droplets by ensuring accurate atomisation into droplets of the desired size.

Can conventional sprayers be used?

As discussed above, conventional mist blowers can be used with CDA attachments. However, the most widely used sprayer in the Caribbean, the hydraulic knapsack, cannot be used for CDA or for oil-based formulations. The battery operated spinning disc sprayers designed for CDA are very cheap and easy to operate, but training in their use will be required.

Who will produce the fungi?

In Brazil, Colombia and Costa Rica there are already small-scale commercial enterprises producing *Metarhizium* and *Beauveria* for use in the coffee and sugarcane industries. Where the demand exists, it is well within the capacity of local enterprise to meet it. This is a great advantage of mycopesticides compared to their chemical counterparts; the technology for production is not complex and is very suitable for small-scale business.

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