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Bugs beware

Here comes the good fungus

An oil-based formulation containing the *Metarhizium* fungus has been developed to control plague locusts.

Robin Taylor introduces a biopesticide being trained to control all manner of irksome insects.

If insects have nightmares, one of their worst must involve being attacked by a fungus such as *Metarhizium*. But for farmers whose crops have been ravaged by locust plagues, and householders worried about chemical used on termites, the fungus offers an environmentally benign form of pest control.

Hundreds of strains of *Metarhizium* occur naturally in Australian soils and many thousands more are found worldwide. Different strains are active against different insects.

During the past 10 years, researchers at CSIRO Entomology, led by Dr Richard Milner, have tested strains of *Metarhizium* against a range of insects. Together with a commercial partner, they are now developing a product for termite control which it is hoped will be on the market in about two years. Field trials against some species of sugarcane grubs, as well as locusts and grasshoppers, are at an advanced stage, and promising results have been achieved with other sugarcane pests and crickets in pasture.

Fungal diseases can directly penetrate the cuticle or skin of an insect, unlike other 'biopesticides' – such as *Bacillus thuringiensis* (Bt) and viral insecticides – which must be eaten and so are only effective against leaf-eating insects.

The *Metarhizium* fungus inhabits the soil as spores. These spores attach to the cuticle of susceptible insects and penetrate into the body where they multiply, filling the insect with hairlike threads known as hyphae.

Below: A sugarcane scarab killed by *Metarhizium* showing the growth of new green spores on the outside.

Left: Healthy sugarcane scarabs attacking the roots of sugarcane.

Once the insect is dead, if conditions are warm and moist, the fungus grows back out through the cuticle and forms a layer of new green spores on the outside of the insect. These spores will persist for several years in the environment (such as in soil) if protected from extremes of heat and ultra violet light.

An important part of Milner's work has been in unravelling the genetic diversity in the fungus using genetic fingerprinting techniques. His laboratory has one of the largest collections of living cultures of *Metarhizium* in the world.

Milner says the vital first step in developing a biopesticide is finding an isolate of the fungus which is highly infectious for the particular pest. These virulent strains are often selected from those found attacking the pest in nature.

This was the case with the Australian plague locust and wingless grasshopper. Under natural conditions the fungus is extremely rare and does not have a significant impact on pest numbers. Control is achieved by bombarding the pest with a massive dose of spores.

'To be useful as a biological insecticide strains must be highly virulent,' Milner says. 'Such strains may be uncommon and in the case of some insects they may even be non-existent.'

Once an effective strain is isolated, it has to be mass produced, formulated as a spray or bait, and the application rate determined by extensive field testing.

'People ask why we don't genetically engineer *Metarhizium* to make it more virulent,' Milner says.

'But it is not that simple. Overseas researchers have produced genetically modified strains, but have found that they donít reproduce on the host because they kill it too quickly, so environmental persistence is not as good.

'They may also harder to mass produce and register with the National Registration Authority; and we don't have

good genes to put into *Metarhizium* to make it super pathogenic.'

One of the attractions of *Metarhizium* is that the infectious spores can be reproduced on simple, cheap substrates.

Although Milner and his colleagues now have collaborators to produce the large quantities of spores required for field trials, they have grown millions of spores in their laboratory on sterilised rice in reusable plastic bottles. Each bottle can produce enough spores to spray one hectare for locust or grasshopper control.

The manufactured spores can be used directly as a natural granule or formulated as a dust, wettable powder, oil concentrate, or other type of granule. For termites, which live in a moist environment, only a few grams of dust is needed to kill entire colonies.

The type of formulation depends on many things, including economics, the target pest and the storage needs. The pure spores, properly dried, can be stored cool for one to two years or for several months at room temperature.

Fungi require moist conditions to grow and *Metarhizium* requires about 24 hours of very high humidity for its spores to infect an insect. Dry conditions, or high temperatures, limit the spread and effectiveness of the fungus and rigorous testing of formulations is needed to overcome these barriers.

Plague protection

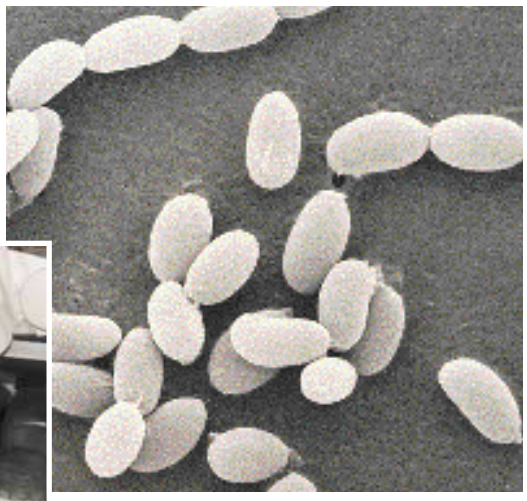
Since 1993 Milner and his colleagues have been successfully testing a strain of the fungus *Metarhizium flavoviride* to kill the Australian plague locust and the wingless grasshopper. For the first time, the technology will allow landholders to shift away from using chemical sprays such as fenitrothion. But the main potential user is the Australian Plague Locust Commission.

In collaboration with the commission, Milner has carried out large-scale field trials in the White Cliffs and Cooma districts of New South Wales and in Queensland. The results of these trials are being used to further develop the formulation and to determine the effectiveness of the spray over a range of conditions. The aim is to release a commercial product in the next few years.

To overcome the dry environment, the researchers have developed an oil-based formulation which the commission can apply directly onto plague hotspots using its normal aerial spray techniques. Using satellite data as well as information from ground surveys, the commission can predict where locusts are most likely to require control.

Bolow: Millions of *Metarhizium* spores have been grown by CSIRO on sterilised rice in plastic bottles.

Right: A scanning electron microscope image of *Metarhizium.*



Ideally, *Metarhizium* should be applied when insects are at the nymph stage as part of a preventative control strategy. *Metarhizium* sprays kill too slowly for use against adults that are already damaging crops, but the infection can prevent locusts from developing the fat required for migration and egg laying. Whereas fenitrothion kills locusts in one to three days, *Metarhizium* takes 10 to 14 days or more, depending on weather conditions. Fortunately, treated insects feed less and less as the disease takes hold.

Another target for control with *Metarhizium* is the sugarcane scarab, the major pest of sugarcane in Australia. The worst is the greyback canegrub, which is found around the Burdekin and Ayr districts of Queensland where chlorpyrifos, the only registered insecticide for its control, may be ineffective.

In small-scale trials by the Bureau of Sugar Experiment Stations and local growers, supported by CSIRO, the Sugar Research and Development Corporation (SRDC), and Bio-Care Technology P/L, *Metarhizium* has consistently reduced numbers of greyback canegrub. Cane tonnage and sugar yields have increased by about 30% in two trials where grub numbers were relatively high. Large-scale trials to test the efficacy over a wider range of conditions are in progress.

'We have very good isolates for the greyback canegrub, negatoria canegrub, locusts, grasshoppers, termites, and crickets, but not for other canegrubs, soldier fly (another pest of sugarcane), and peanut scarab,' Milner says.

His work in collaboration with the Victorian Department of Natural Resources to control crickets in western Victorian pastures has shown that treatment with *Metarhizium* can reduce cricket numbers by 70-80%. This is similar to the 80% control achieved with the chemical insecticide, malathion.

Milner says despite the obvious environmental benefits of biopesticides such as *Metarhizium*, people are not prepared to pay more for them.

'We are trying to produce biological insecticides that are as effective as chemical insecticides and as cheap,' he says. 'To do this we need isolates that can be mass produced, are very infectious and will give a high level of control when introduced at levels that farmers can afford.'

There is still much research to be done with *Metarhizium* to explore its remarkable features. Other target pests include house flies and leaf-eating pests of plantation eucalypts.

An adult spur-throated locust feasts on sorghum in Queensland. Information from satellites and ground-surveys is used to predict hotspots of locust infestation.