FUNGI TO CONTROL PESTS IN THE SOIL

'Myco-insecticides' are showing promise as a clean replacement for chemicals in some pest control applications

You can't miss insect pests that nibble the leaves of trees and crops, causing destruction in farms, gardens and city parks. Less obviously, though, pests in the soil can quietly gnaw away at roots, seriously weakening a plant both nutritionally and structurally.

The larvae of scarab beetles are prime examples of soil-dwelling insect pests in Australia. (In addition, some adults in this family can cause problems by their leaf-eating.) Peanuts, potatoes and sugar-cane all suffer from various species of scarab larvae, also called white grubs. The larva of the soldier fly attacks sugar-cane roots too.

The problem is not trivial. Losses from larvae of scarabs and soldier fly and the cost of insecticides used to control them add up to more than $5 million every year. Losses of peanut crops come to about $2 million a year. As well as these crop pests, other grubs attack the roots of pasture grasses, and the termites found in many areas of the country can target the wood in our houses.

All such pests are good candidates for biological control by means of fungi that are adapted to live in the soil, an environment with a constant high humidity — which the target insects also need. Several fungi in our soils already attack these insects; the first step on the road towards devising an effective biological control agent, therefore, involves selecting for the most deadly.

Once that is done, and we can grow and apply the chosen fungus, we have the beginnings of a 'myco-insecticide'. Why bother? Until recently, farmers controlled soil pests by means of chemical insecticides: unfortunately, these lasted a long time and, because they were applied to soil, tended to find their way into groundwater. Consequently, many of the formulations used — being based on the infamous organochlorines — were banned for use in this fashion in 1987. Since then, one of the main control methods has been an organophosphate insecticide, in the form of slow-release granules. But this is expensive, it is not at its most effective in clay soils, the larvae may become resistant and opposition to any chemical methods of pest control on food crops is growing in the community and government.

So Dr Richard Milner and his team at the CSIRO Division of Entomology are busily researching myco-insecticides for insect soil pests and, with the collaboration of the Bureau of Sugar Experimental Stations, have already come up with a possible answer to the infestations that plague the sugar-cane fields of northern Queensland.

In Australia, three species of the soil fungus *Metarhizium* are known to occur. These can infect and kill a wide range of insects. The fungi produce millions of tiny green spores called conidia. In the soil, these attach to the insects' bodies, where they germinate, producing a 'holdfast' and a tube that, by secretion of the right enzymes and mechanical pressure, can penetrate directly through the hard insect cuticle. Hyphae — fungal branches or tubes — spread out inside the host, some free-floating in the fluid of the body cavity, absorbing nutrients and growing. Eventually, their sheer mass kills the insect, which has become a firm shell stuffed full of a fungal mat (mycelium).

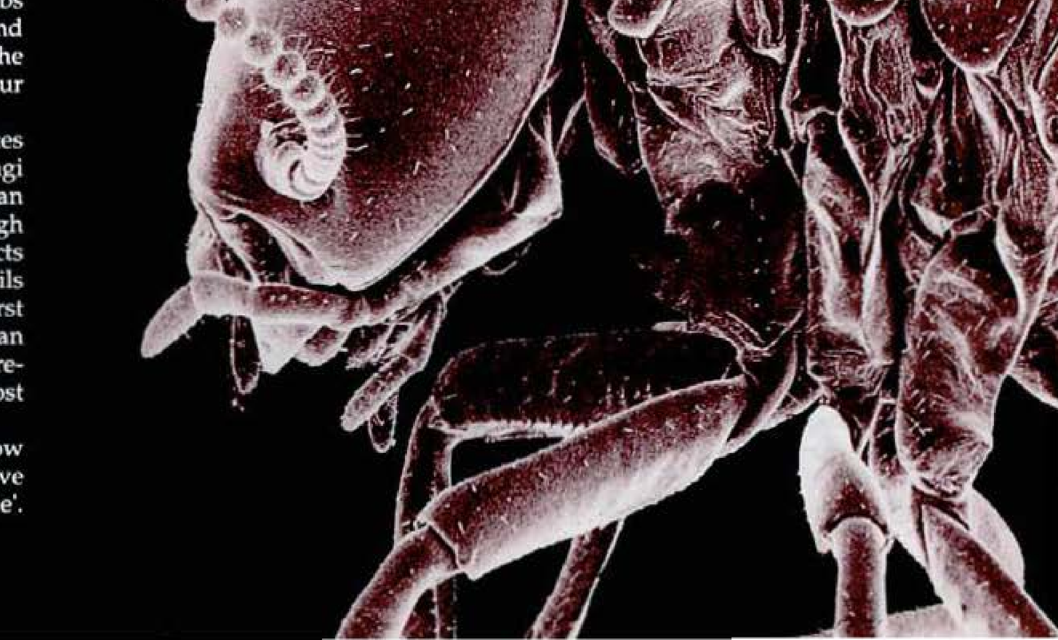
After the host's death, the fungus grows out through the cuticle as a white mycelium. Eventually it produces a mass of green conidia at its surface, and these disperse away from the rotting corpse, staying dormant (for years in the right conditions) as they await contact with another insect.

The conidia can be eaten, and usually pass through the gut of both suitable hosts and other creatures unharmed and without germinating. This helps in their dispersal. However, infection around the mouthparts of insects, onto which conidia acquired during feeding can stick, does occur.

The fungus grows quite readily in the laboratory, feeding on nutrients in agar, but in Nature it appears not to live that way — for example, on de-

Portrait of a pest: a scanning electron micrograph of a scarab larva.

Microscopy Centre, CSIRO Division of Entomology



Richard Milner

The effect of the fungus on a scarab grub — before (top) and after (lower), dead and covered with a coat of grey conidia that will spread the fungus.

caying vegetation or animal matter — but rather requires a living insect, which it must then kill in order to produce its conidia. It is thus a pathogen (disease-causer), and not a saprophyte — able to live on scavenged nutrients from the remains of living things in the environment — as are so many other soil fungi.

Needless to say, soil-dwelling insects have spent millions of years in the company of such fungi, and they have ways of fighting back. For example, some insects may produce enzymes (phenoloxidases) that interfere with the ability of the fungus to manufacture its cell walls and hence grow. The concentration of these enzymes in the haemolymph (blood) of the insect increases after a conidium has germinated but before the complete penetration of the germ tube through the cuticle. Evidently, some signal 'warns' the insect that an invasion is in progress.

But, of course, the battle can escalate. Some strains of fungi can produce substances, termed destruxins, that somehow interfere with the production of, or render ineffective, the insects' defensive enzymes. Quite possibly further details of each side's armoury remain to be discovered, but the point is that various degrees of effectiveness exist, depending on the strain of fungus and the type of insect.

Insect pathologists have known for many years that one species of *Metarhizium* would be very effective as a control agent — namely *M. anisopliae*. By taking samples from soil around the country and from many different infected insects, Dr Milner and his colleagues have found that the species varies considerably in Nature. It's no good, therefore, just gathering the fungus, identifying it, applying it and then hoping for the best. The road to success is paved by the artful selection of the right strains of *M. anisopliae*. And strains must not only kill effectively, they must also be easily cultivated, produce large numbers of conidia and be able to persist in the soil.

Of the three main sources of strains — the existing culture collections of laboratories around the world, the soil and infected insects in the field — the last has provided those most useful against pest insects. Surprisingly, strains from the soil are often ineffective, perhaps because they are specific pathogens of other soil-dwellers, or possibly because some are genuinely saprophytic, a feeding strategy that has been described in other parts of the world.

Dr Milner and his team have collected and tested more than 100 isolates (fungal cultures) from infected larvae or soil beneath sugar-cane. They were looking for a strain that had a high 'kill rate', that produced conidia in large numbers and that survived well in the soil conditions prevailing in the area in which it would be used.

The first test of a fungal isolate was to roll pest larvae in vast numbers of dry conidia — not quite the way in which an infection would take hold in the field, but it nevertheless gave an idea which were good killers. Only those that killed all larvae in less than 2 weeks passed this first hurdle, and were put to a second test.

For the next test, the team put conidia in water at doses of either 100 million or 1 million per mL, and dipped larvae into the water. Good isolates would kill all larvae within 2 weeks at the higher dose of conidia, and at least half at the lower one.

The final test came closest to modelling soil conditions. The scientists added conidia to the soil at known doses, either by mixing them in evenly or by putting conidia-coated rice grains into the soil. For a strain to be effective the lethal concentration required to kill half the insect larvae (the LC50) had to be less than 100 000 conidia per gram of soil. The best strains achieved 50% kills with only 10 000 or even 1000 per gram. But because infection takes place much more slowly in soil, the destruction of half of the pests would often take up to 7 weeks to occur.

This exhaustive selection process finally yielded three strains highly patho-

This close-up shows how the fungal hyphae (threads) grow out through gaps in the insect cuticle.

John Roger

Left: A grub emerging from a destroyed peanut.

Below: Dr Richard Milner exposing scarab grubs (white) in the soil at the base of sugar-cane plants.

Richard Bull

genic for sugar-cane scarab pests. Of course, the effectiveness of a myco-insecticide depends on other factors too. Apart from an assumed high humidity — without which the larval pests themselves would not be present — the most important is temperature.

Most strains of *M. anisopliae* grew well between 20 and 30°C. (However, they can survive short periods at much higher temperatures — about 40°C — as found in soil.) The three most effective ones were well adapted to the temperature profile of the soil in the Queensland sugar-cane fields. Other isolates of the fungus — from Victoria and Tasmania — can infect and kill a pest at temperatures below 10°C, and are therefore being investigated as control agents for a temperate-pasture pest.

Other factors that can impinge on the success of the fungus include soil pH, organic matter, other microbes and saturation with water. Conidia can germinate over the pH range of most soils, but too much water excludes oxygen and thereby prevents sporulation (the formation of conidia) in infected dead insects. However, periods of such severe waterlogging are likely to be brief. Dr Milner's field observations and laboratory studies suggested that organic matter in the soil favoured the development of the fungal disease in insects.

The age of the insect host also plays an important part in determining the effectiveness of the fungus. Biologists in the field have observed that the final larval stage is the most often infected, partly because it is the most mobile and the longest-lasting stage and therefore stands the greatest chance of encountering conidia. The pupae and adults are rarely infected, and the eggs even less often.

Dr Milner has found that most strains of *M. anisopliae* are very specific to particular insect hosts. This is ideal for a myco-insecticide, as it means that beneficial insects will not be harmed. However, it has the drawback that where a complex of pest species exists, as with sugar-cane, then it may be necessary to use more than one strain to bring about full protection.

Working in collaboration with the Bureau of Sugar Experimental Stations, Dr Milner and his team have conducted numerous field experiments with a range of fungal strains on cane crops in Queensland. Conidia don't move down into the soil very effectively, so the best strategy to get the desired fungus in place is to add it when the crop is planted. The scientists have discovered that the conidia survive best when coated onto an inert carrier substance.

In the case of crops like sugar-cane, the pests in the soil take at least a year after planting to build up to a sizeable population. Thus the fungus, applied during planting, must remain as viable conidia in the soil for 12 months or more. Dr Milner's latest findings suggest that this is not a problem.

Accordingly, commercialisation of the effective strains is proceeding apace. The Division of Entomology has granted Incitec Pty Ltd the licence for the strains developed for sugar-cane pests. The company will work on how to produce the conidia in commercial quantities as well as the marketing of the myco-insecticide.

Readers may wonder whether a possible 'insect in the ointment' in this control strategy is the development of resistance to the fungus. Of course, some degree of resistance is likely to develop eventually — but, unlike the case with chemical insecticides, the control agent will itself be able to change, and if it does not do so fast enough then new strains can be selected.

Moreover, chemical insecticides usually have a very narrow target — often just one enzyme — in the pest. For resistance to appear 'all' that is required (although it may take decades) is a suitable mutation to the affected enzyme that renders it able to operate despite the presence of the chemical. Combating the process of fungal infection, by contrast, involves the operation of many biochemical pathways and the participation of different cell types. As a result, resistance would probably take longer to evolve and would be less effective, mimicking the state in Nature where, over time, neither the pathogen nor the host species achieves a total victory.

Meanwhile Dr Milner, in collaboration with Divisional colleague Dr Tony Watson, is turning his attention to the control of termites. As these are social insects — vast numbers living together in mounds, with a surprising ability to sacrifice individuals in order to combat threats to the society as a whole — they raise rather different problems. However, first results have suggested that the right strain of *M. anisopliae* would do the trick, provided we adopt the right strategy. But that's another story...

Roger Beckmann

More about the topic

The selection of strains of *Metarhizium anisopliae* for control of Australian sugar-cane white grubs. R.J. Milner. In 'The Use of Pathogens in Scarab Pest Management', ed. T.A. Jackson and T.R. Glare. (Intercept Publishers: Andover, U.K., 1991, in press.)

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Recent progress with *Metarhizium anisopliae* for pest control in Australia. R.J. Milner. *Proceedings of 1st Asia/Pacific Conference of Entomology, Chiang Mai, November 1989* (in press).