Hormonal bind for **bad insects**

In the 1960s, Harvard University insect biologist Carroll Williams informed the world that only 0.1% of insects were pests. The remaining species were innocuous, even beneficial to humans.

Williams made his point in response to the emerging problems of indiscriminate pesticide use. Chlorinated insecticides such as dieldren and heptachlor, and organophosphates such as parathion had promised to revolutionise crop protection, but in two decades had caused environmental contamination, residues in humans and animals and pesticide resistance.

As a means of reducing these harmful side-effects, and protecting the innocent insect species, Williams suggested developing a new generation of pest-specific insecticides based on the chemistry of the insect's own hormones.

Nearly 40 years on, scientists at CSIRO Molecular Science, in collaboration with DuPont and Melbourne's Biomolecular Research Institute, have adapted Carroll Williams' idea. They have embarked on a project to develop new insecticides that target the receptor for the insect steroid hormone ecdysone.

By targeting the ecdysone receptor the scientists intend to overcome a previously unforeseen problem with hormone-based insecticides.

'A major problem with Carroll Williams' approach is that insects have a system of breaking down their own hormones once they have performed their regulatory function,' CSIRO molecular biologist Dr Ron Hill says. 'We're trying to develop new chemistries that will interact with the insect ecdysone receptor, but can't be broken down by the insect's catabolic systems.'

Ecdysone and its receptor help to control insect growth and development. Normally, the concentration of ecdysone rises and falls many times during the insect's life cycle. When the hormone binds its receptor in the nucleus of the cell, it activates genes that regulate metamorphosis, reproduction and moulting. Synthetic molecules that interact with the receptor, but resist catabolism, will switch on the genes controlling these events at the wrong time.

'So the insects will moult prematurely or they'll undergo metamorphosis ahead of time, causing major disruption to their orderly process of development,' Hill says.

And according to CSIRO organic chemist Dr Paul Savage, the targetted control such as synthetic molecules offer will ensure greater environmental friendliness.

'Some of the pesticides used in the past were very dirty and very nasty, because they were broad spectrum and tended to hit biochemical pathways that many organisms have,' he says. 'The benefit of this approach is that it targets a receptor in moulting invertebrates that is absent from humans, birds, fish and so on. So toxicity will be limited to the targetted pest species.'

Signals and crystals

Hill and Savage are tackling the project in two ways with the help of CSIRO molecular biologist Dr Gary Hannan and protein chemist Dr Lloyd Graham.

The first approach tests the interaction of natural or synthetic molecules with the receptor in cultured cells.

To do this, genes encoding the ecdysone receptor are inserted into cells along with a 'reporter gene'. When a test molecule binds the ecdysone receptor, the reporter gene is switched on and produces a signal such as a change in colour or antibiotic resistance. This signal tells the scientists their test molecule has worked and they can single it out for further study.

The second approach is being investigated together with Dr Peter Colman of the Biomolecular Research Institute. It involves making crystals of the receptor protein in order to determine its structure by a technique called X-ray crystallography.

Earlier work has shown that the ecdysone receptor is made up of two protein sub-units called EcR and USP (see diagram below right). These proteins interact to form a number of regions or 'domains', including the 'ligand-binding' domain that binds ecdysone. This ligand-binding domain can be 'fooled' into binding other ecdysone-like molecules, if, like a lock and key system, they fit the shape of the domain precisely.

If good receptor protein crystals can be made, X-ray crystallography will allow the scientists to see the structure of the

Above: Receptor-targetted insecticides have the potential to control specific pests such as aphids and sheep blowfly.

Right: This simplified model of part of the ecdysone receptor binding site shows a domain from the ultraspiracle protein (USP, pink) and a domain from the ecdysone receptor protein (EcR, blue). A hormone molecule (yellow) has been modelled into the ligand binding site. On binding hormone, the EcR domain changes its conformation from that shown to one where the hormone is trapped in the binding site.

ecdysone-binding domain and design synthetic molecules that mimic ecdysone and fit its binding site exactly.

Both methods are being applied to the study of ecdysone receptors from a number of insect groups, including the Diptera (flies) and Hemiptera (plant sucking bugs). These groups are commercially important as they include destructive pests such as the sheep blowfly and aphid.

The scientists also plan to compare the diversity of the ecdysone receptor genes in each insect group, to determine the potential for taxon-specific insecticides. They have already found evidence at the molecular level to indicate that ecdysone receptors from the different groups of insects studied vary significantly.

'We know there are significant differences between insect receptors at the DNA sequence level which encode amino-acid sequence variations in the ligand-binding regions of the ecdysone receptors,' Hill says. 'These differences should be enough to allow the development of insecticides that kill only specific insect groups.'

This is good news for non-target insects. 'By targetting the ecdysone receptor of a specific insect, such as southern corn root worm, or aphids, or buffalo fly, we could design an insecticide that would bind that receptor, but not those of beneficial insects such as lady beetles,' Savage says.

He says receptor-targetted insecticides have the potential to be used in many areas where pests are involved, but particularly in the commercially important areas of crop protection, veterinary chemicals and human parasites.

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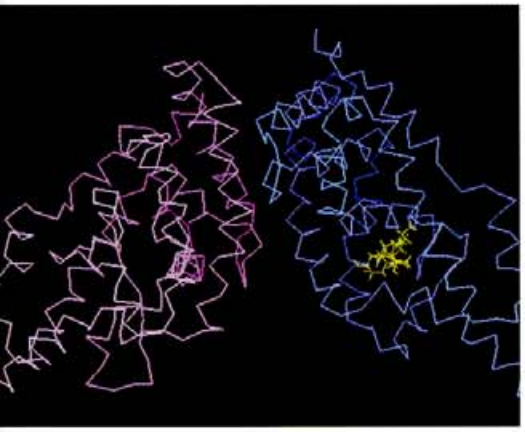

A termite covered with the *Metarhizium* fungus.

Friendly fungus has **termites** covered

A FUNGAL insecticide developed by CSIRO entomologists offers a new weapon to counter the termite's destructive reign over man-made structures.

The fungus, *Metarhizium anisopliae*, produces tiny spores that kill termites by penetrating the insect's cuticle and filling its body cavity with hair-like threads called hyphae. When the termite dies, the fungus returns to the body surface and produces new spores.

Hundreds of Metarhizium strains occur naturally in Australian soils and termite mounds, and their virulence towards termites and other insects varies. A research team led by Dr Richard Milner at CSIRO Entomology has identified among them several that can kill destructive termite species.

The most promising strain, FI–610, grows in temperatures of 30–36°C – the temperature of termite nests – and is safe for humans, animals and the environment.

Early field tests with FI–610 showed that when the spores were used like a dusting powder and blown directly into the nursery region of termite mounds, colonies of more than one million individuals could be killed in less than three months.

But the nests of destructive termites, which may be small or hidden in trees, are sometimes difficult to find.

To get around this problem, Milner is developing a remote baiting system in collaboration with Seed, Grain and Biotechnology Australia, and with funding from the Forest and Wood Products Research and Development Corporation.

The system will borrows from a strategy used in cockroach baiting, but must confound the natural ability of the termite to detect and overcome pathogens and chemical pesticides.

'Termites are clever enough to detect threats to their colony,' Milner says. 'When we put the fungus in one part of a mound only, the termites wall off this area and do not enter it. And fungus-killed termites are mostly found at the base of a colony in a walled off "cemetery", effectively quarantined from the healthy colony above. So the fungus must be very carefully introduced.'

To do this, Seed, Grain and Biotechnology Australia is field testing FI–610 and other promising strains in special bait formulations designed to mask the presence of the fungal spores.

The resulting product is likely to be used as part of an integrated control system that includes chemicals and changes to the environment surrounding susceptible homes and other structures.

Milner hopes to have the fungus approved by the National Registration Authority and on the market in the next two years. If he succeeds, *Metarhizium* will provide Australia's first biological weapon against termites.

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