An alternative to chemical pesticides

Without pesticides, the prospects for continuing to feed the world's rapidly growing population would be bleak. However, concern about the serious environmental consequences that their use can bring has prompted a search for new alternatives.

Chemical residues from some insecticides persist in the ecosystem, with traces finding their way into wildlife, livestock, and people. Many insecticides are indiscriminate in their effects, so they destroy 'good' insects — those that feed on pests or have other important roles in the natural ecosystem — along with the 'bad'.

We need a way of targeting specific pest insects with sprays that have no effect on other species, and leave no hazardous residues. Scientists at the CSIRO Division of Entomolgy are working on one possibility.

Dr Peter Christian and Dr John Oakeshott and their team are targeting bollworms — caterpillars of the genus *Heliothis* — which cause damage costing Australian crop farmers an estimated $450 million a year. Bollworms will happily eat cotton, maize, sorghum, tomatoes, peanuts, and other valuable crops. And they don't just eat the leaves; they'll feed on seed heads and other parts. The adult moths, however, do no damage at all.

Bollworms have been a target of 'chemical warfare' for decades, with some unfortunate results.

For one thing, toxic chemicals have been accumulating in the environment; for another, the pests have started to develop resistance. A novel approach is clearly timely, and the one that the CSIRO team is investigating involves a specially fortified virus.

Viruses are, of course, tiny parasites of cells, and insects are as susceptible to infection with them as we and all other organisms are. The virus attaches to the cell by means of molecules on its outer coat that latch onto complementary receptor molecules on the cell membrane. Once latched on, the virus can enter the cell, where its nucleic acid (carried within its protein shell) gives instructions to the cell's enzymes to produce millions of copies of viral nucleic acid and protein coats. The cell's own life processes are disrupted and usually it dies — but not before it has served its viral hijacker and manufactured more particles to infect neighbouring cells.

The killer

It so happens that a group called the nuclear polyhedrosis viruses (NPV) infect *Heliothis* caterpillars in the wild and can indeed kill them. The younger the caterpillar the more susceptible it is. Also, younger individuals need a lower infective dose — that is, it doesn't take as many individual virus particles to establish a deadly infection.

Adult moths are not susceptible to the virus, and in the last stage of the caterpillar before it pupates the infection proceeds so slowly that the insect will usually succeed in pupating and becoming an adult without succumbing.

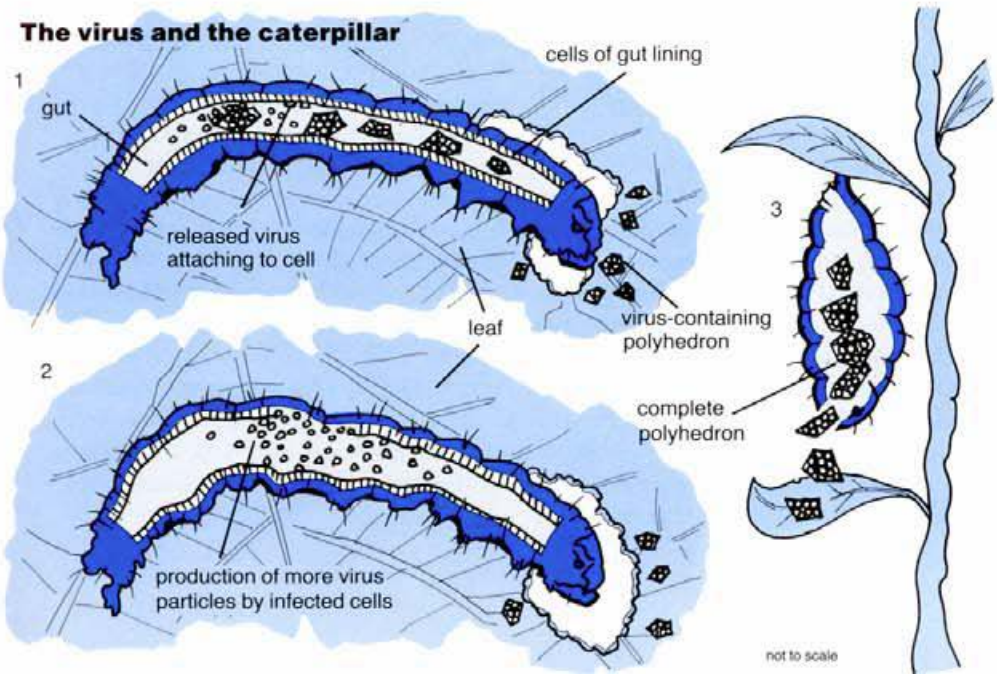
When the virus first infects a caterpillar, its nucleic acid follows the pattern described above and directs production of new virus particles that infect other cells; but after about 18 hours, the viral DNA starts to direct the production, in vast amounts, of a protein called polyhedrin.

New virus particles find themselves embedded in this protein within the cell. Hundreds of viruses in this protein matrix form a structure 1–2 μm in diameter termed a polyhedron (so-called because it is many-faceted).

Starting with the lining of the intestinal tract, cells in the infected caterpillar break down, and the sick insect climbs to the top of a stalk and attaches itself there, while the virus continues to do its work. Eventually, nearly all the cells apart from the skin are destroyed and the hapless creature is nothing but a bag of virus-enriched 'goo'. Usually this bursts, scattering polyhedra onto leaves and soil below.

The polyhedron matrix surrounding the individual virus particles protects them from the ultraviolet radiation of sunlight, which can damage nucleic acid molecules. Without such shelter, NPV viruses can only survive for a few hours outdoors. Embedded in the ball of polyhedrin protein, they remain viable for a few days at least and, if they are out of the light altogether, can survive for 3–4 years.

Caterpillars browsing on plants ingest polyhedra. In the gut, these dissolve, releasing the individual virus particles to infect the cells of the lining, and the cycle starts again. How long the virus takes to kill its host depends on the temperature, but at 25°C most caterpillars survive for about 5 days after infection. Lower temperatures extend the period. During this time, the caterpillars carry on eating, so the virus naturally present in the field already doesn't help very much in curtailing damage to crops.



After a caterpillar ingests a virus-containing polyhedron, virus particles are released and infect cells of the gut lining (1). The infected cells release more virus particles (2). Later in the infection the protein polyhedrin is produced, and virus particles become embedded in it. Eventually polyhedra scatter onto surrounding leaves and the soil when the bloated caterpillar corpse bursts (3).

Trojan horse

If NPVs are so slow-acting, one may well ask 'Why are Dr Christian and his colleagues working on them as potential viral insecticides?' The answer is, because it's possible to use the virus as a means to get a fast-acting toxin into the caterpillars. Such a poison must, of course, only affect insects and be completely innocuous to vertebrates — that is, mammals, birds, reptiles, amphibians, and fish — although the fact that the virus cannot reproduce in their cells gives them natural protection. Similarly, the safety of other insects that may be susceptible to the toxin is also assured because the virus can only reproduce in closely related *Heliothis* species.

The toxin needs to be a protein, and the information directing its manufacture must be encoded in the virus's DNA. Infected insect cells, obeying the instructions carried by the virus, would manufacture it along with the other viral components.

They would thereby sign their own death warrants even more surely than they do by merely reproducing the virus, for the toxin that they manufacture, while it need not necessarily damage the individual cell, will kill the whole caterpillar.

In the early 1980s, scientists in the United States, knowing that the polyhedrin protein (although protective) is not essential for infection, removed the gene for it from NPV DNA. Having succeeded in this, they then inserted in its place genes for other products — among them human molecules such as insulin — that the caterpillar cells duly produced.

Part of the reason for choosing to remove the polyhedrin gene was that the protein is produced in abundance, which implies that the gene has a very effective promoter. (A promoter is a stretch of DNA that lies next to a gene and controls its expression.) Thus, the polyhedrin promoter causes any foreign gene adjacent to it to be transcribed frequently, ensuring that its product is made in large quantities. The problem is that the lack of polyhedrin makes the altered virus useless in the wild.

What type of molecule would be an effective toxin for the caterpillars? Overseas scientists cunningly suggested an enzyme (harmless in itself) that, by digesting an important hormone, would make the insect stop feeding and give rise to premature development, causing it to pupate early. Because of their small size, such premature pupae would fail to turn into adults, or would give rise to stunted moths that could not reproduce effectively. Unfortunately, although the virus was successfully engineered, the idea has not yet worked in practice because the enzyme didn't remain stable *in vivo*.

The caterpillar on a cotton boll.

A wide range of other possible 'toxins' remains. The insects do not need to die; a molecule that paralyses them would effectively prevent them damaging crops. Dr Christian and his colleagues have a number of molecules in mind, which they will stitch into the viral DNA without removing the polyhedrin gene, so ensuring that the engineered virus can survive for the necessary time in the field. They will duplicate the polyhedrin gene-promoter, and place the toxin gene next to this second promoter, so achieving the production of large amounts of toxin without removing the polyhedrin-producing capacity of the virus.

The scientists have established a system for keeping *Heliothis* cells in culture, to provide a means of replicating viruses. They have also collected various strains of the virus from the field, and selected one that showed good disease-causing ability. The team is currently working on identifying and isolating the polyhedrin gene and its promoter in this strain of virus. Later, they will infect cultured insect cells with the virus and the DNA for the chosen toxin and await 'recombination' to bring about a new virus that carries the toxin gene. Biochemical means will allow the scientists to identify which of many cells have produced the desired recombinant virus.

The future

Farmers may not be spraying the Division's engineered virus onto their crops for at least a decade. The research, assuming it succeeds, will be followed by development of the product by ICI Ltd (under an agreement entered into in 1989). That product must then pass stringent safety tests relating to the release of genetically modified organisms before commercial production can begin.

Dr Christian expects a successful viral insecticide will kill caterpillars within 24 hours. It should be no more expensive than current chemical ones, and should be every bit as effective in terms of its kill rate. Of course, the time of its application will be important, and it will be vital that farmers use it circumspectly to avoid the risk of resistance developing. (Over-use of many chemical insecticides in various parts of the world has led to a faster than necessary development of resistance.)

Naturally, the idea of viral control invites comparison with myxomatosis. But the myxoma virus was not present in Australia until scientists released it as a biological control agent and then left it to do its work. The *Heliothis* virus is already present here, and the genetically engineered form may need to be regularly applied to crops at the crucial time. Its population will not build up to a sufficient level in the wild for it to act effectively at the right time of year.

Rabbits now have considerable resistance to myxomatosis and, as mentioned, the possibility exists that *Heliothis* will similarly develop some resistance to the virus. It is hoped that careful use of the insecticide, sometimes in conjunction with other treatments, will maximise its useful life.

Many other insects may also be amenable to control by the application of genetically engineered insect viruses. The Division of Entomology, in collaboration with scientists in the Division of Biomolecular Engineering, is also working on a group of insect viruses called entomopoxviruses.

If all this work comes to fruition as planned, the persistent, undiscriminating, and noxious chemicals of the past will slowly become redundant — good news for the environment.

Roger Beckmann

More about the topic

The potential of genetically engineered baculoviruses for insect pest control. P.D. Christian and J.G. Oakeshott. *Australian Journal of Biotechnology*, 1989, **3**, 264–6.