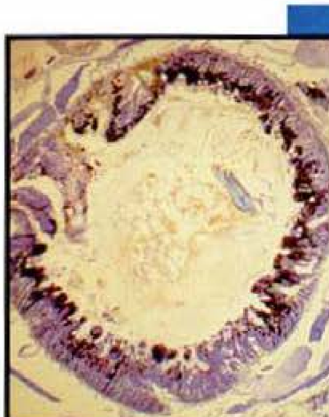
'Turbo-charged' viruses speed insect kill

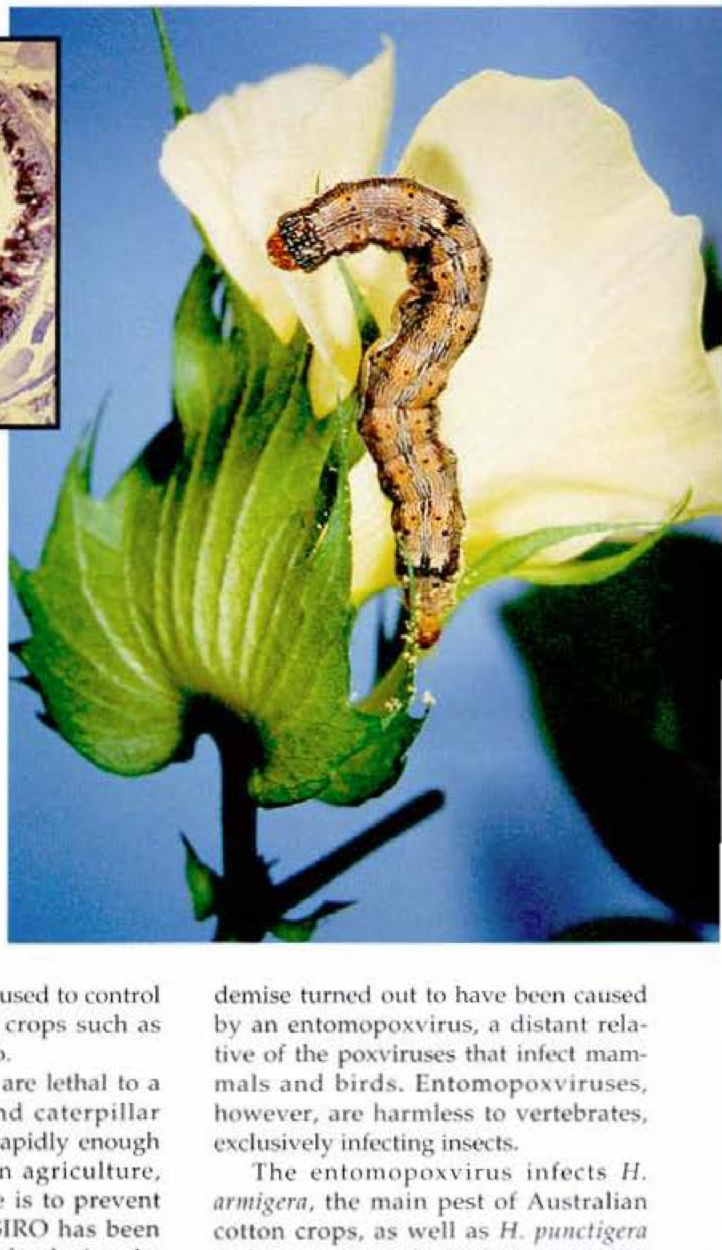
Researchers at the CSIRO Division of Entomology are experimenting with specialised insect-killing viruses that could substitute for chemical sprays. Early experiments have focused on increasing the potency of a baculovirus, nuclear polyhedrosis virus (NPV). While baculoviruses are highly infectious, they take a week or more to kill. During this period, the insect may continue to feed, causing crop damage. The challenge is to reduce the kill time to 24 hours, comparable with conventional chemical sprays that kill within minutes to hours.

Dr Peter Christian's research group is exploring the possibility of harnessing the infectious potential of NPV, using it as a delivery system for fast-knockdown toxins or other molecules that disrupt the insect's life cycle. Baculoviruses are good candidates for genetic surgery because with 100 DNA-encoded genes, they are among the largest of viruses, and their genomes can readily accommodate extra genes.

The idea of using viruses to control insect pests is not new. The agrochemical company Sandoz marketed a non-recombinant NPV insecticide in the 1970s, but it could not compete with the new generation of synthetic pyrethroids. Another non-recombinant baculovirus is used in Germany to control codling moth in apples.

American and British researchers have been studying a strain of NPV that infects the moth *Autographa californica*, a common pest of fruit and vegetables in the US. Although it has a wide host range, *Autographa* NPV is ineffective against *Helicoverpa* caterpillars, and has the additional drawback of infecting the South American *Cactoblastis* caterpillars that have kept prickly pear under control in Australia for 60 years. The Division of Entomology, however, is focusing on *Helicoverpa* NPV. This NPV only infects insects in the genus *Helicoverpa* and *Heliothis*, many of which are among the world's most damaging insect pests and includes the cotton boll worm. Up to 40% of the world's chemical insecticides are used to control these insects that infest crops such as cotton, maize and tobacco.





Right: The cotton boll worm, (*Helicoverpa armigera*) the main pest of Australian cotton crops.

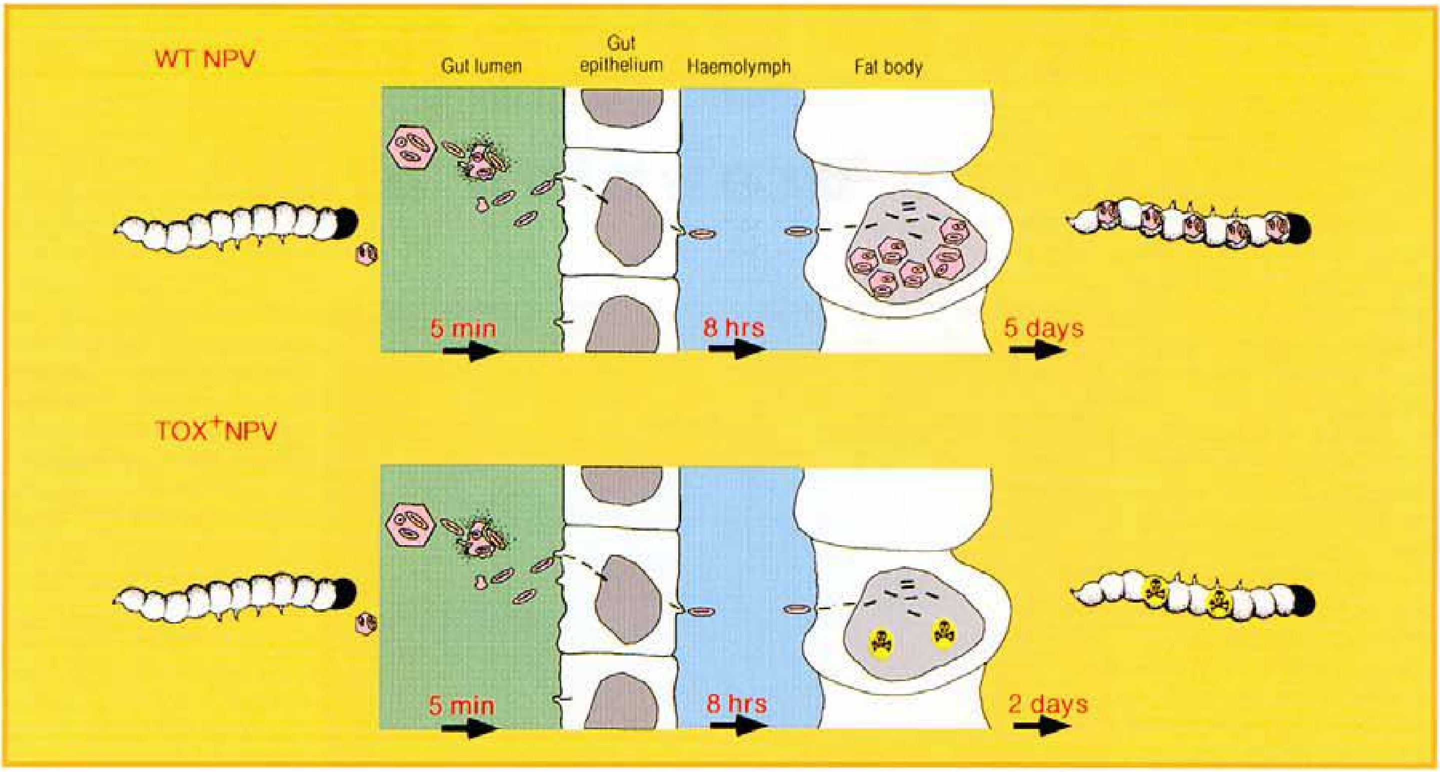
Above: A cross-section of an infected *H. armigera* larvae stained for the presence of *H. armigera* stunt virus shows only midgut cells arranged in the large circle to be infected. The lack of the dark stain in the other tissue outside the midgut and inside the insect illustrates the specificity of the virus.

While baculoviruses are lethal to a wide range of moth and caterpillar larvae, they do not kill rapidly enough to be of practical use in agriculture, where the first objective is to prevent damage to the crop. CSIRO has been studying the possibility of reducing the virus's kill interval with toxin genes from other insects, or even an anti-feedant compound that would switch off the caterpillar's voracious appetite.

Dr David Dall's research group has been studying another virus, isolated by Dr Bob Teakle from the Queensland Department of Primary Industries from a smear of tissue which constituted the remains of an unidentifiable dead caterpillar in Queensland. The caterpillar's demise turned out to have been caused by an entomopoxvirus, a distant relative of the poxviruses that infect mammals and birds. Entomopoxviruses, however, are harmless to vertebrates, exclusively infecting insects.

The entomopoxvirus infects *H. armigera*, the main pest of Australian cotton crops, as well as *H. punctigera* and more distantly related caterpillar species. Again, the strategy would be to 'turbo-charge' the virus with toxin genes which enhance its speed of kill.

What might seem to be a duplication of research effort is part of a broader strategy against *Helicoverpa* cotton pests. Dall says that alternating the different viruses in the field will make it more difficult for the insects to evolve resistance to any single virus. The industry does not need a repeat of



The wild-type virus normally takes between five and six days to kill its insect host. When the whole process is broken down it takes about five minutes for the insect to ingest the virus and for the virus to enter the host cell and establish an infection. After about eight hours the virus has made more copies of itself and new cells are infected. After this it takes several succcessive rounds of viral replication before the insect succumbs to the viral infection. With recombinant viruses however, the above process is short-circuited by expressing a toxin in the second and subsequent rounds of replication. The toxin paralyses the insect earlier than the virus would normally take the wild-type virus to overwhelm the insect (which it does by sheer strength of numbers), leading to the insects 'death' in three to four days.

the resistance problems encountered with chemical insecticides. As a generic package, the toxin genes would also offer some economies of scale in production, minimising the cost of the new biocides.

Dr Terry Hanzlik and Dr Karl Gordon are exploring an alternative approach, which would exploit another recently-discovered virus as a ready-made, fast-knockdown insecticide. In 1988, a mystery infection began wiping out colonies of *Helicoverpa* caterpillars at the Division of Entomology. A virus susequently been isolated from these colonies has since been named *Helicoverpa armigera* stunt virus (HaSV).

With only three genes, HaSV is among the smallest and simplest of all RNA viruses. One gene encodes a capsid protein which assembles into particles that home in on the cells lining the insect host's midgut, and spirit the virus's RNA genome inside. The infected cells die and slough off, preventing the insect absorbing its food. Hanzlik suspects that a second gene, encoding the replicase enzyme that makes copies of the viral genome, induces a phenomenon called apoptosis, in which the cell deliberately commits suicide. This is a strategy that may have evolved to contain the infection.

Hanzlik says HaSV is highly specific, growing only in the midgut cells of *Heliothis/Helicoverpa* caterpillars. The particles made from the HaSV capsid protein are highly stable, resisting digestion in the insect's gut, a property which could make them an ideal vector or delivery system for fast-killing natural toxins.

It may not even be necessary to incorporate a toxin. A key property of the HaSV virus is that it rapidly causes caterpillars to stop feeding. 'The caterpillar comes along, eats the self-replicating toxin and stops eating very rapidly,' Hanzlik says. 'As far as efficacy is concerned, we're getting into the territory of chemical insecticides. These properties make HaSV an ideal candidate for a fast-kill insecticide.'

Hanzlik and his team are exploring a cheaper and potentially more effective approach than using the virus as an external spray. That is, putting the the entire genetic blueprint of the virus into the plant, so that viral particles would be synthesised in the leaves.

They have already succeeded in expressing the entire HaSV blueprint ino protoplasts (naked plant cells which have had their cellulose walls stripped away). Together with Dr Phil Larkin's team at the neighboring Division of Plant Industry, they are now pursuing the next step: to insert the HaSV genes into the chromosomes of plant cells and then induce regeneration of adult plants able to make their own anti-insect virus.

Because the experiment involves transferring more than two thirds of a virus's genome, the division sought approval from the Genetic Manipulation Advisory Committee, Australia's genetic engineering watchdog. GMAC approved the experiment, subject to security precautions, but any proposal to release transgenic plants into the environment would require review.

The attractions of virus-based insecticides are manifold: they are specific to a narrow range of insect hosts, harmless to mammals, birds, reptiles and fish, do not concentrate in food webs and do not pollute the environment. If they could be integrated within crop plants, they would eliminate the costs associated with spraying. While no recombinant viral insecticide has yet been tested under field conditions, there is optimism that they will prove considerably more durable than chemical insecticides in the face of insects' enormous capacity to develop resistance.

Graeme O'Neill