Green genes join war against insects

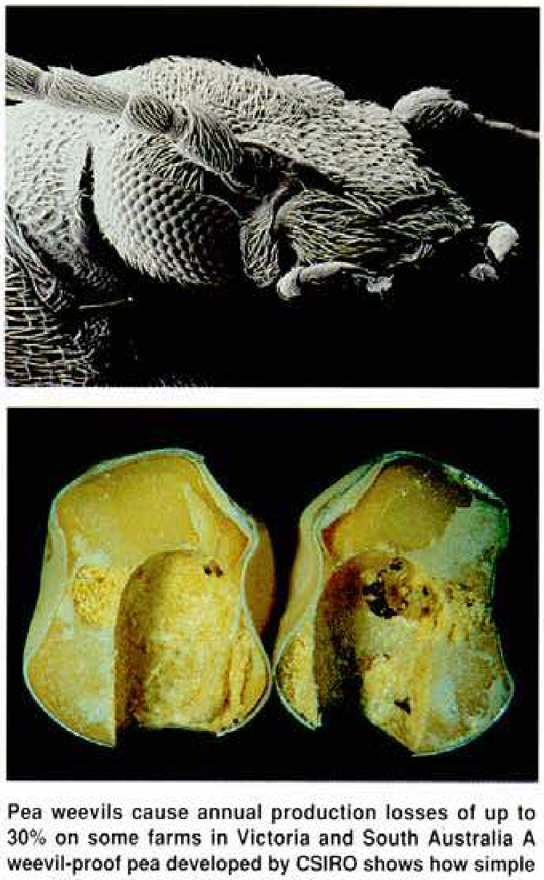
Growers will benefit from a new approach to insect control as scientists develop crops, viruses and bacteria to replace chemicals. **Graeme O'Neill** reports.

Humans and insects were competing for food long before farmers first domesticated wild plants on the Mesopotamian delta and in New Guinea's highlands some 10 000 years ago. Even the potent insecticides of the 20th century, however, have offered humans only transient gains over these persistent adversaries.

The world still forfeits up to a quarter of its annual agricultural production to insect attack, to insect-transmitted virus diseases, and to pests that infest stored grain. But Australia and other western nations are now moving into a new era of gene-based defensive weapons which could turn the tide of this perennial battle decisively in humanity's favour.

Farmers, consumers and the environment should benefit from cleaner, 'greener' production as farmers adopt transgenic varieties of familiar cereal, vegetable and fruit crops. (A transgenic organism has a transferred gene incorporated into the chromosomes of all its cells). The plants will not be obviously different from traditional varieties – the changes typically will involve only one or two genes – but they will have far-reaching impact on plant and animal production.

Agriculture will rely less on toxic chemical sprays as plants acquire their own inbuilt defences, based on nature's own insecticides. Researchers are also adding genes to enhance the food value, storage life or processing properties of agricultural produce, and even to improve the flavour or aesthetic appeal of traditional foods (see story page 12).



Pea weevils cause annual production losses of up to 30% on some farms in Victoria and South Australia A weevil-proof pea developed by CSIRO shows how simple genetic surgery might lift world food production.

A weevil-proof pea developed by CSIRO's Division of Plant Industry in Canberra exemplifies how simple genetic surgery might lift food production in a hungry world. With funding from growers through the Grains Research and Development Corporation, a team led by Dr TJ Higgins has transplanted from the common bean (*Phaseolus vulgaris*) into garden peas, a gene which protects peas against attack by the pea weevil. Pea weevils cause annual production losses of up to 30% on some farms in Victoria and South Australia, where most of Australia's garden and field peas are grown.

Many weevils and other seed-eating insects secrete a starch-digesting enzyme called alpha-amylase in their digestive tract. The borrowed bean gene encodes a protein which confers natural protection against weevil attack by inhibiting this enzyme. Unable to digest their food, the larvae starve to death soon after hatching, and before they can mature into adult weevils and breed.

Higgins says weevils and other insect pests can cause enormous damage to stored cereal grain and grain legumes. He says the bean gene is now being introduced into field peas, which are dried and used in animal feeds.

Higgins has sent some of the transgenic peas to Dr Larry Murdock of Purdue University at Indiana in the United States. Murdock has confirmed that the gene also works against the cowpea and azuki bean weevils, major pests of stored grain legumes in Asian and African nations. The CSIRO group also plans to introduce the gene into chickpeas, a dietary staple in Asia, and believes it could be useful in cowpeas, mung beans, lentils and other starch-containing seeds.

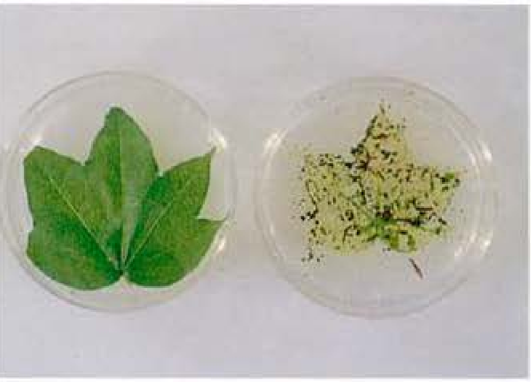
Another gene-transplant performed by Higgins and his colleagues – this time from sunflowers into narrow-leafed lupins – should bring benefits for the poultry and pig industries.

The sunflower albumin gene encodes a high-sulfur protein which will compensate for low levels of the amino acid methionine in many feed mixes. Australia imports more than $12 million worth of raw methionine, grown in bacterial cultures by Japanese biotechnology companies.

The Plant Industry team already has a second-generation transgenic lupin whose seeds contain up to 5% of sunflower albumin by weight, enough to help optimise the amino acid pattern of the mixes fed to poultry, pigs and sheep.

Because the amino acid pattern of dietary protein is less than ideal, livestock waste some of their protein intake. The addition of this one gene to lupins should lift the efficiency of livestock production. With the gene now established in narrow-leaf lupins, the Cooperative Research Centre for Mediterranean Agriculture in Perth will use conventional breeding to develop commercial varieties for Western Australian farmers.

Above left: A group led by Dr Danny Llewellyn is trialling transgenic cottons designed to counter *Helicoverpa*, the industry's most resilient pest.



Left: The cotton leaf on the right has been eaten by a cotton boll worm.

Natural insecticides

Genetic engineering has opened up a range of novel routes to counter insect pests and the virus diseases they transmit. The technique closest to practical use involves transplanting modified genes from the common bacterium *Bacillus thuringiensis* directly into crop plants. The genes encode natural insecticides which protect the plants against leaf-chewing caterpillars.

Strains of *B. thuringiensis* have co-evolved with many different insect hosts, so that variants of the so-called Bt proteins tend to be lethal to particular groups of related insects but not to others. The toxins home in on receptor proteins on the surface of the cells lining the insect's gut, killing the cells and preventing the insect from absorbing food. Weakened, stunted larvae usually die of secondary bacterial infections.

Dr Danny Llewellyn from the Division of Plant Industry says there are at least six generic groups of Bt toxin, each adapted to different insect orders. These subdivide further, according to the type of receptor the toxin targets. Bt toxins are lethal to insects, but harmless to birds and mammals.

Researchers at the US-based agrochemical company Monsanto have cloned several variants of the Bt gene, replacing the DNA switches that control their activity with DNA sequences cloned from plant genes. With these plant-specific DNA switches, called promoters, molecular biologists can control both the level and site of expression of the Bt genes in plant tissues. In CSIRO's transgenic cottons, the toxins are synthesised in the plant's leaves and other structures, providing a deadly meal for caterpillars.

Llewellyn's group recently began field-trialling transgenic varieties of CSIRO's own Siokra and Sicala cottons, containing Bt genes licensed from Monsanto. The cry IA (c) and cry IIA variants of the Bt gene are designed to counter the Australian cotton industry's most destructive and resilient pests, *Helicoverpa punctigera* and *H. armigera*.

In the early 1970s, pesticide-resistant *H. armigera* drove the cotton industry out of the Western Australia's Ord River irrigation scheme. The moths were able to breed year-round in the Ord's sub-tropical climate and as insecticide use increased, resistant populations rapidly emerged.

Inevitably, the Bt genes will also impose selection pressures for resistance, but the novel arrangement of the genes, along with complementary management strategies, should prolong the field life of the transgenic cotton cultivars. Eventually, each plant will actually contain two different Bt genes, challenging the pest to develop resistance to two different toxins simultaneously.

Hatching larvae must eat to survive, and with the pesticide being synthesised within the cotton plant, Llewellyn says *Helicoverpa* cannot avoid it, whereas pesticides delivered as sprays may miss their target. The pest is also at its most vulnerable stage, leaving fewer survivors able to found resistant populations.

In the long-running battle between humans and insects, the prolific reproductive capacity of the pests has always given them the edge. Another strategy to manage resistance will be to maintain refuges for Bt-susceptible insects in areas around cotton crops, so called 'refugia', which would regularly re-infuse pest populations with susceptible genes to dilute the emergence and spread of Bt-resistance genes.

Dr Gary Fitt of the CSIRO Cotton Research Unit says a computer model of the dynamics of insecticide resistance developed by Dr Rick Roush of Cornell University in New York indicates that resistance can be managed using refugia. Roush has estimated that the major lepidopteran pest of vegetable crops in the US, the diamond-backed moth, could take at least 95 and as many as 44 000 generations to break through resistant plants carrying two Bt genes in conjunction with refugia.

Data on the dynamics of resistance are sparse, so the model involves some guesswork, but even for a sub-tropical insect producing a dozen generations a year, it suggests that resistance to the Bt toxins could take decades to centuries to emerge in transgenic cotton crops with the proper management strategies. The issue of insect resistance will need to be explored with other crops as well.

Potato leaf roll virus can cause yield losses of up to 30% in some Australian potato-growing areas. Right and below: A single potato and a market garden display the devastating impact of the virus. Far right: Dr Peter Waterhouse's research team has introduced a gene that is resistant to potato leaf roll virus into two varieties of crisping potatoes. Below right: The scientists will soon be analysing the results of their latest field trials involving more than 3000 transgenic potato plants.

Protecting potatoes

The economic loss in cotton results from physical damage to the crop's flower buds and bolls, but losses in many other crops are due to aphid-transmitted virus diseases.

In 1990, Dr Peter Waterhouse's research team at CSIRO, in a joint project with the Smith's Snackfood company, introduced a gene into two crisping varieties in potato, cv Kennebec and cv Atlantic, to control potato leaf roll virus which can cause yield losses of up to 30% in some Australian potato-growing areas.

These transgenic plants were first tested in the field in 1991 at a research station at Gatton in Queensland. As this trial was the first of any transgenic plants in the field in Australia, it tested only 90 transgenic plants and was concerned with evaluating their agronomic performance in the absence of the virus and measuring the possible spread of potato pollen (and therefore transgenes) into other crops.

Since then there have been two other similarly small trials, one at Gatton, again, but in the presence of the virus, and the other at Crookwell in NSW. Both trials gave promising results, but the real test will be when scentists analyse the results of a five-state field trial, conducted with the state departments of agriculture. The trial involves plots containing a total of more than 3000 transgenic plants.

At all the sites, transgenic and non-transgenic plants will be grown side-by-side and their yields measured. Waterhouse and colleagues expect a natural virus infection at a site in the Atherton Tablelands in Queensland; they have manufactured virus infection at sites in Victoria, Queensland and South Australia by inter-planting with virus-infected plants; and they have sites where the virus should be absent. The tubers from the virus-free site in Queensland will be due for harvest in December and will be processed by the Smith's Snackfood Company into crisps for taste-testing.

The resistance gene for potato leaf-roll virus is derived from the virus itself: it encodes the protein that forms the virus's coat. When an aphid with virus-laden saliva sucks sap from the potato leaf, it injects virus particles into the leaf cells. For reasons that remain unclear, the virus cannot replicate in cells containing the gene for the synthetic capsid protein.

'We think two things may be going on,' Waterhouse says. 'The gene may not be working at the protein level at all, but at the level of the RNA messenger.'

When a gene is activated, its code is copied into a single-stranded molecule of nucleic acid, called a messenger RNA (mRNA), which instructs the cell's factories to synthesise the encoded protein. Even if the gene is incomplete, preventing the plant cells from synthesising the virus capsid protein, it still protects the plant.

Waterhouse suspects the explanation lies in the fact that the cell tightly controls the level of mRNAs in its interior, using specialised enzymes called ribonucleases to destroy those surplus to requirements. When a plant virus deploys its own single-stranded RNA genetic code, including the instructions for its own capsid protein, into a cell already oversupplied with similar capsid protein mRNAs, it may be broken down by ribonucleases, halting the cycle of replication.

More about genetic engineering

Larkin P (1994) *Genes at work: Biotechnology.* CSIRO Australia.