Can we eliminate the pests that ruin crops but leave their benign relatives unharmed? **Bijal Trivedi** explores a smart new strategy

It's a bug's death

THE environmental movement is often traced back to *Silent Spring*, the 1962 book in which Rachel Carson documented some of the harmful effects of pesticides on people and animals, and called for stricter controls. Half a century on, the book remains controversial but there is now no doubt that however useful pesticides are, they cause an immense amount of damage too.

Pesticide poisoning is a major health problem. Every year thousands of farm workers die of pesticide poisoning and millions more suffer severe effects, mainly in developing countries. A survey in Nicaragua last year concluded that 2 per cent of the country's adult population suffers pesticide poisoning annually. This kind of finding is not unusual.

Then there are the environmental effects. Many pesticides are toxic to a wide range of animals, and dose is often the only factor that restricts the killing to insects. They kill beneficial insects alongside harmful ones, which means that once farmers start using pesticides they often have to keep using them because there are fewer natural predators to help control pest populations. Some pesticides persist in the environment for decades and accumulate up the food chain. Plants genetically modified to produce biodegradable insecticides such as Bt are one way to solve these problems, but this approach does not work for all pests and there is intense opposition to GM crops in many countries.

Now, however, researchers are working on an entirely new generation of pesticides, one that promises to target individual species while leaving other animals unharmed. These could be sprayed onto plants like conventional pesticides or genetically engineered into crops. Already, one company is preparing for field tests of a spray targeting the Colorado potato beetle, a major pest (pictured, right).

The key to the new pesticides is gene silencing, and RNA interference (RNAi) in particular. In 1998, it was discovered that when a double strand of RNA (dsRNA) matching the sequence of a particular gene is injected into nematode worms, that gene gets switched off, or silenced.

RNAi may have evolved as a defence against viruses containing double-stranded RNA. Now, though, all plants and animals rely on RNA interference to help control the activity of their own genes. Remarkably, the effect can spread from cell to cell in some invertebrates: when dsRNA is injected into one part of the body, the matching gene often gets silenced in other parts of the body too. Just as surprising was the discovery that simply feeding dsRNA to worms could silence genes, although it was not as effective as injection. That result caught the attention of researchers like Steven Whyard, then working for Australia's national insect research institute, CSIRO Entomology, in Canberra. "We thought: it won't work in an insect, but let's give it a try anyway because if it does work we're off and running with something rather interesting," he says.

"Invasive pests are a serious problem – genetic tools that attack specific species would make for very powerful weapons"

It did work, Whyard and his colleagues at CSIRO patented the method, and by 2006 its pesticidal potential was becoming clear. A team led by Richard Newcomb at Plant and Food Research in Auckland, New Zealand, showed that gene activity in the guts and the antennae of the light brown apple moth, a major pest in Australia and New Zealand, could be greatly reduced by feeding them dsRNA.

A year later, two landmark papers published together in *Nature Biotechnology* proved that the effect was strong enough to protect plants from pests (vol 25, p 1231). One team, led by James Roberts of Monsanto in Chesterfield, Missouri, first fed a variety of dsRNAs to western corn rootworm larvae to see if any killed them. They found the most effective RNA targeted a gene coding for the enzyme v-ATPase. Next, the team genetically modified maize to produce this dsRNA in its roots. The modified plants suffered less root damage when infested with rootworm.

A second team, led by Xiao-Ya Chen, now head of the Shanghai Institutes for Biological Sciences at the Chinese Academy of Sciences, tried a less direct strategy. Cotton plants produce a natural pesticide called gossypol. Pests like the cotton bollworm, however, have evolved resistance, and Chen's team has found that this resistance depends on an enzyme called cytochrome P450. When bollworm larvae were fed plant material containing gossypol as well as dsRNA targeting the gene for cytochrome P450, the insects stopped growing and started dying.

These studies generated a lot of interest, but big questions remained. One is the effect of dsRNAs on humans (see "Is it safe?", page 36), and the other concerns specificity. If the dsRNA is designed to target a gene sequence that ›

IS IT SAFE?

Using gene silencing, or RNA interference (RNAi) to target specific pests while leaving other species unharmed sounds like an enormous step forward. But can we be sure that the key ingredient – double-stranded RNA (dsRNA) – won't have unexpected side effects in people?

Although most RNA in cells is single-stranded, all plants and animals also produce dsRNA to regulate the activity of their own genes. "There are lots of dsRNAs in the plant and animal products that we eat every day," says Michael Czech, a molecular biologist at the University of Massachusetts Medical School in Worcester, who is exploring ways to use RNAi to treat type II diabetes. "Those RNA molecules are rapidly chopped up by the enzymes in our gut and are non-toxic."

There is also a second line of defence in the form of enzymes in our blood that break down dsRNA. "You could inject dsRNA into primates at moderate doses and nothing would happen," says Daniel Anderson of the David H. Koch Institute for Integrative Cancer Research at the Massachusetts Institute of Technology, who is designing drugs based on RNAi.

Even without these defences, the long dsRNAs used for killing insects are difficult to transport into human cells and if they did get inside they could trigger an immune defence mechanism rather than shutting down specific genes, although it's not yet clear if this in itself might be harmful. In vertebrates, short pieces of dsRNA around 21 base-pairs long, called small interfering RNAs or siRNAs, are more easily used to silence genes. In fact, the big problem for those trying to develop drugs based on gene silencing is finding ways to get siRNAs into cells in the human body (*New Scientist*, 27 February 2009, p 15).

So there is good reason to think sprays containing dsRNAs lethal to insects, or plants modified to produce them, will pass all the safety tests. However, if the RNA was altered in a way that allows it to get into human cells, perhaps by making it persist longer in the environment, it might cause problems. "If you modify the dsRNA – by encapsulating it or changing the RNA molecule – then you are imposing a new chemistry that could have toxic effects on humans," Czech cautions.

If medical researchers ever devise an effective way to deliver siRNA orally, it might be possible to use it for controlling a wide range of animals. "If they can figure ways to deliver it to all organisms, then it could be used to control all kinds of invasive species," says Steven Whyard of the University of Manitoba in Winnipeg, Canada. As long as the siRNAs are carefully designed, only the target species should be affected.

It's not much of a leap to wonder whether RNAi could also be abused to attack specific groups of people or even individuals. There have long been concerns about the misuse of RNAi. "Like any technology, it can be used for good or bad," says Whyard. "Yes, this technology could be used to target individuals, but I don't know how effective it would be. It's probably a whole lot more trouble than the current methods people use."

is unique to a particular species, then in theory that dsRNA should be harmless to other species. But this assumption had not been tested.

Whyard, now at the University of Manitoba in Winnipeg, Canada, took on the challenge. First, he tried targeting four quite different species – fruit flies, pea aphids, red flour beetles and tobacco hornworms – via the gene for v-ATPase, which in the gut is a protein pump which controls acid levels. "If you mess that up, you will cause malfunctioning of the gut physiology and the insect may ultimately starve or die," says Whyard.

Whyard designed four different dsRNAs, each one specific to the v-ATPase gene variant in one of the four species. When fed the dsRNA matching their genes, between 50 and 70 per cent of the insects died. When a dsRNA designed for one species was fed to the other three species, less than 5 per cent died.

Next, Whyard tried to kill four closely related species of fruit fly of the genus *Drosophila*. This time he targeted a gene for gamma-tubulin, which is essential in cell division. When he fed the fruit flies dsRNA targeting their specific gene variant, between 35 and 55 per cent died. In contrast, a dsRNA designed for one fruit fly species had little effect on a non-target species, killing less than 5 per cent. "I was quite pleased to see that," says Whyard. "Actually I was surprised."

Because there are so few differences in the sequences of the gamma-tubulin gene variants among the fruit flies, Whyard had expected more "off-target" deaths.

As more and more genomes are sequenced, it might become possible to find genes that are only present in a single species. Targeting these would reduce the effects on other species still further, Whyard says. So far, though, his research is the only work of its kind. Much more needs to be done to prove dsRNA's effects really are species-specific.

Whyard's work also shows that simply spraying dsRNA onto insects' food can be enough to kill them. Stored grains could be coated in dsRNA to protect them from pests like the red flour beetle, he suggests, although spraying it on crops like conventional pesticides might be a different matter. Devgen, a biotech company based in Ghent, Belgium, is ready to field test a spray containing dsRNA targeting the

SIMON FRASIER/SPL

Silencing crucial genes could kill pests such as (clockwise from left) cotton bollworms, pea aphids and tobacco hornworms

KRISTA QUEENEY/SOLENT NEWS/REX FEATURES

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Colorado potato beetle, though with patent and research papers pending, head of technology development Geert Plaetinck will only say that the spray looks very promising.

Devgen has also investigated ways to kill that much-despised household pest, the cockroach. It tried targeting seven genes and found that silencing four of them individually proved lethal. But – surprise, surprise – the roaches proved tough to kill, Plaetinck says, requiring much higher doses of dsRNA than other insects.

Spraying will only be a viable approach if dsRNA can be cheaply mass produced, especially as the dsRNA will probably break down faster than conventional pesticides. It would also work only for pests that eat the parts of the plant that are above ground. For those like the corn rootworm, plants would have to be engineered to produce dsRNAs. The specificity of gene silencing means it could be especially useful for targeting invasive species, which are very hard to kill using conventional methods without causing a lot of collateral damage.

"I can't imagine anything better," says James Carey, an entomologist at the University of California, Davis. "Invasives are a serious problem and tools that target specific species could be a very powerful weapon." Still, tackling widely dispersed invaders like the light brown apple moth, which was recently discovered in California and feeds on a wide range of plants, would be tricky. The dsRNA would have to be coupled to some kind of bait to lure the moths, Carey says.

It might not even be necessary to kill pests directly. "If you silenced a pheromone receptor, you could block communication and mating," Newcomb points out. Interfering with the social structure of insect colonies could also curb populations, says Michael Scharf at the University of Florida in Gainesville, who has silenced genes in termites by feeding them dsRNA. He has shown that silencing a gene called *Hex-2* triggers the transformation of workers into soldiers and thinks that boosting the number of soldiers might cause a termite colony to collapse. "The soldiers are the slackers in society," says Scharf. "So more soldiers is bad for the colony. The workers can't feed, clean and manage a colony that contains more than about 10 per cent soldiers."

Non-lethal ways of controlling pests should, in theory, slow the evolution of resistance, which is becoming a massive problem with conventional pesticides and will also eventually happen with crops containing pesticide genes. Insects could acquire resistance to specific dsRNAs too, by evolving new variants of the gene that the dsRNA targets. But if this happens, the dsRNA could be changed to target the new variants. Such tweaks would be much easier and cheaper than developing new conventional pesticides.

It would be rash to say resistance could be completely ruled out, however. "I'm always reluctant to say that insects won't mount resistance," says Whyard. "Insects have mounted resistance to everything we've thrown at them so far." Nevertheless, because the cellular machinery required for dsRNA to silence genes is essential for normal development, mutations that block the effect of dsRNAs at a fundamental level should be lethal to the insect.

Other uncertainties remain, too. For example, the approach might not work for all insects, or even for all genes. One recent study found that feeding dsRNA to tsetse flies will shut down a gene in gut cells but not in fat cells. There may be many other complications of this kind.

Nevertheless, the potential benefits, and profits, are certainly getting attention. "Every agrochemical company in the US is interested in RNAi – it's on everybody's radar," says Scharf. ■

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