Mothra Meets Its Match

Researchers engineer insect-killing viruses

By TINA ADLER

A naturally occurring virus infected and killed a beet armyworm larva, a common agricultural pest.

American Cyanamid

If insects had their own tabloids, the headlines these days might read: "Man-made viruses will eat your guts out!" or "Young larvae left paralyzed from supervirus — human tampering suspected."

Viruses that destroy insects have existed in nature for eons. For more than 60 years, scientists have tried to sic these microbes on crop pests. Viral insecticides, however, rarely pack the punch of their chemical counterparts or compete in the costs arena.

So researchers are tweaking them genetically — taking out a gene here, adding one there — hoping to increase their killing power. The new strategy might intimidate even Mothra, the giant moth that attacked Tokyo in the 1962 film of the same name.

One company, American Cyanamid, recently submitted plans to the Environmental Protection Agency to do a field test in the U.S. of a virus loaded with an extra gene that produces a substance toxic to some insects. This would be the first such experiment in the United States.

These new insecticides are impressive, promising, and — some scientists and environmentalists say — frightening. Indeed, protests similar to those that arose over a bovine growth hormone (SN: 12/15/90, p.372) and the genetically engineered Flavr Savr tomato (SN: 5/28/94, p.342) may greet the outdoor testing of these assassin microbes.

The bugs that destroy crops are themselves daunting. A typical agricultural pest increases its weight 10,000-fold in 20 days as it dines in a farmer's field. The bigger they grow, the more they eat.

Even the most virulent naturally occurring viruses take 4 to 7 days to destroy their insect hosts. So speeding up a virus' killing time ranks as a primary goal of the handful of research teams working on this novel approach to pest control.

P. Hernandez Crespo

Researchers in England test their viral insecticide on cabbages growing outside in nylon-covered cages.

American Cyanamid Co.'s agricultural research division in Princeton, N.J., is hoping to get EPA approval to begin field testing an autographa californica multicapsid nuclear polyhedrosis virus (AcMNPV) into which company scientists have inserted a scorpion gene. AcMNPV belongs to a family of microorganisms called baculoviruses, which kill lepidoptera (butterflies and moths). Most research on genetically engineered insecticides uses this family of viruses.

Laboratory studies show that the scorpion-toxin virus stops some lepidoptera larvae from eating in about half the time it takes the unaltered, wild version, says Cyanamid's Peter M. Dierks.

Before granting approval for the trials to take place, EPA must solicit public comments. The agency can expect to receive numerous responses, researchers say.

The company's proposed test "does not satisfy environmental and ecological concerns," asserts H. Alan Wood of the Boyce Thompson Institute for Plant Research in Ithaca, N.Y. "They are going to spray it out on the field with no limits," yet they don't know what else the virus may infect, he says.

U.S. scientists have field-tested baculovirus from which a gene had been removed. And last year, British investigators began an outdoor trial of a virus containing a scorpion gene.

The gene added by both American Cyanamid and the British groups produces a toxin that kills only insects. The toxin, found in the venom of scorpions, paralyzes larvae, preventing them from eating.

American Cyanamid researchers hope to run one field test this fall and 13 others next year, each on a one-fifth-acre plot. They plan to spray the virus on lettuce and cotton suffering from natural infestations of cabbage loopers or tobacco budworms, both moth larvae. The experiments would take place in Bridgeton, N.J., and Maricopa, Ariz., and each would last 3 to 4 weeks, Dierks says.

The company would surround each plot with a buffer zone but does not plan to use any other method to contain the virus, Dierks says. "We feel the virus is intrinsically safe or we wouldn't [plan to] be out there and EPA wouldn't allow us to be out there," he argues.

Nor will the company test to see if the virus could transfer the scorpion gene to other viruses. It considers that an unlikely possibility, since the viruses would have to simultaneously infect one insect.

For the past 2 years, American Cyanamid scientists have been field-testing an AcMNPV from which they had deleted a gene that prevents infected insects from molting and pupating. Since insects stop eating while they molt, removing the gene ensures that larvae infected with the modified virus will eat less than bugs infected with wild virus.

Insects infected with the engineered version caused noticeably less leaf damage than insects infected with the wild type, Dierks says. He suspects that the virus beefed up with the scorpion-toxin gene will perform better than the one missing the antimolting gene.

Researchers at the National Environment Research Council's Institute of Virology and Environmental Microbiology in Oxford, England, began field-testing their scorpion-toxin virus last year. They find it kills the cabbage looper faster than the wild type, says lead investigator David H. L. Bishop. However, the modified virus has not performed as well in the field as it did in the laboratory, the team reports in the July 14 NATURE.

Even in their outdoor experiments, the British scientists keep their cabbages and virus at arm's length from nature. They enclose the 1-meter-square plots with a net and fence to ward off everything from birds to insect interlopers. Before spraying the genetically engineered virus on the plants, they line the net with paper to trap stray virus particles. So far, they have not detected any of the virus outside the enclosures.

Despite these precautions, the study has met with considerable opposition in England over the last year. Citizens fear we "are creating a supervirus," says coauthor Jennifer S. Cory. "A lot of people worry about these things. We are trying to approach it quite cautiously."

The whole viral insecticide endeavor raises numerous concerns. Before giving their support to genetically engineered insecticides, scientists and regulators should ask whether these possibly high-risk molecular methods rank as the best approach, argues Jane Rissler of the Union of Concerned Scientists in Washington, D.C.

"We must not lose sight of the sustainable agricultural methods that may do quite as well as these sophisticated techniques," she argues.

Scientists doing the work question, for example, whether the engineered viruses will infect organisms other than those that the wild version normally invades. Might they destroy beneficial insects, for example?

So far, studies suggest that the modified and wild-type viruses pick on the same insect species. Investigators have studied a number of insects that the engineered and wild viruses infect, but no one knows how many more may play host to the microbes. "And is anyone ever going to know? No," Dierks asserts.

Genetic engineers trying to create viral insecticides rely on baculoviruses in part because these viruses are quite choosy about what they infect. But just how picky remains unclear.

"As [scientists] do more tests, they seem to find the host range [of viruses] is wider," contends Mark Williamson of the University of York in England. The virus used in the scorpion-toxin studies can kill 125 to 250 of the 2,500 species of British lepidoptera, he says.

"I think some of these baculoviruses may infect more pests than we thought," says Boyce Thompson's Wood. With funding from EPA and the U.S. Department of Agriculture, he will soon begin tracking down what beneficial insects a battery of baculoviruses infects.

Stealth infections worry Wood. "It may be that [naturally occurring baculoviruses] infect beneficial insects without producing any signs or symptoms," he warns.

Such invisible invasions would make it difficult to determine accurately the viruses' range of hosts. Moreover, if a virus has a new gene in it, "an inapparent infection is going to become very apparent," asserts Wood.

Researchers have also examined the possibility that engineered viruses might one day outcompete their wild cousins, but that appears unlikely. For one thing, wild baculoviruses produce three to five times as many progeny as the recombinant viruses, says Bruce D. Hammock of the University of California, Davis.

Also, for unknown reasons, larvae infected by wild viruses migrate to the tops of plants, where the larvae eventually become highly explosive bags of viral particles. Insects infected by the engineered viruses usually fall to the ground, where viral particles have less opportunity to disperse. Hammock and his colleagues recently began a greenhouse study that is looking at how quickly a wild type might replace a recombinant virus in a given area.

Debate continues on whether researchers should disinfect sites to kill the virus remaining in the soil after an outdoor experiment, Dierks says.

Upon completion of their tests last year with the virus lacking the molting-prevention gene, American Cyanamid researchers treated their plots with a solution of 1 percent bleach. After 6 months, they saw no signs of the engineered version at any of the sites. Instead of chemically disinfecting the soil, spraying it with the wild virus may also do the job, Dierks contends.

The motivation to develop fast-acting viral insecticides stems in part from the capability of chemical pesticides to poison much more than the targeted pests. Also, some insects are now resistant to these chemicals.

Trevor Williams

Cabbage loopers, unobstructed by insecticides, eat their way through a cabbage leaf.

Scientists wonder whether engineered-virus potions may eventually run into a resistance problem, or help solve it.

"This is an area where we don't have a great deal of data to draw upon," Dierks admits. "It is going to be one of the key things we are going to be following in our testing program."

Insects may develop some resistance to viruses if growers were to apply them in great amounts for 25 to 50 years, says Lois K. Miller of the University of Georgia in Athens. But she and others doubt that viral insecticides will ever see such heavy use.

Hammock's team finds that insects resistant to pyrethroid insecticides don't stand up to a mixture of a pyrethroid and virus engineered to express the scorpion toxin. Moreover, this brew appears to kill insects faster than either of its ingredients does alone.

The first field experiment of a genetically altered virus in the United States, carried out from 1989 to 1991, sought to address environmental risks posed by these high-tech viral insecticides (SN: 7/15/89, p.46). Wood and his colleagues tested a virus modified to survive only a short while in a population of wild baculoviruses, the researchers report in the April 1994 Environmental Entomology.

Their original design proved less than successful. Large amounts of the virus remained in the soil. "And it's going to be there long after I die," says Wood, cofounder of AgriVirion, a biotechnology company with headquarters in New York City.

So the researchers have refined their methods. Using the same baculovirus, AcMNPV, they remove the polyhedrin gene, which forms a protective coat around viral particles. They freeze-dry insects infested with the altered virus, dissolve them in water, and spray the solution on the plants.

In field tests, the virus appears to tolerate the damaging effects of sunlight long enough to attack the cabbage looper. But the virus fails to reproduce, and no virus remains in the soil.

This summer, the EPA told Wood and his colleagues it would no longer require them to seek its permission every time they want to run a small-scale outdoor trial of the polyhedrin-deleted virus. EPA would normally require a 90-day advance notice.

In the laboratory, Wood and his colleagues have also tested the polyhedrin-deleted virus loaded with a toxin gene from the *Diguetia canities* spider. The engineered virus takes less time than the wild type to stop the insect from eating, the team reported in August.

If researchers' efforts bear fruit, farmers and forest managers may eventually have an array of genetically altered baculoviruses to fight lepidoptera pests. Viruses to tackle other unwanted creatures, such as beetles, may also become available some day. But for now, baculoviruses have limited uses.

"You have to have a fairly specific pest problem that you are dealing with in order to use the viruses as pesticides," says Miller. Few baculoviruses do a good job on more than one major pest. "The beauty about baculoviruses is that they are so specific, but that's also their major problem commercially," she adds.

Baculoviruses "are so specific that industry will find it very hard to develop them and find a market," Hammock worries. Production costs will exceed those for chemical insecticides, many believe. But as more insects become resistant to chemical pesticides, alternatives will become more attractive, Miller adds. □