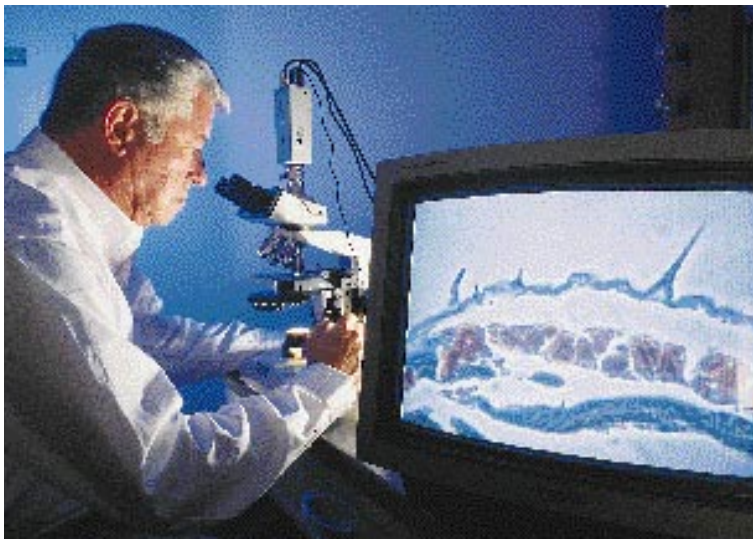
Virus Zaps Pests, Speeds Medical Research

A virus that kills crop-eating caterpillars may soon power a new, environmentally friendly insecticide.

The virus slays tobacco budworms, one of the worst enemies of cotton plants and a ravager of tomatoes and other crops as well. Field and laboratory tests suggest that the microbe also fells dozens of other worm pests. The list includes the alfalfa looper caterpillar, *Autographa californica*—the virus' namesake—along with cabbage looper and beet armyworm caterpillars that feast on cabbage, broccoli, and other vulnerable vegetables in farms and gardens.

KEITH WELLER



Entomologist Patrick Vail observes the internal tissues of a virus-infected cabbage looper larva magnified about 900 times on screen. (K7169-14)

Meanwhile, the virus has already landed a starring role in biotechnology labs around the world. It simplifies and speeds the manufacture of researchable quantities of intriguing proteins and other compounds for scientists in medical, veterinary, and biological research.

The first experimental AIDS vaccine approved for tests on humans in the United States, for example, was created using the hardworking virus. Produced by MicroGeneSys of Meriden, Connecticut, the vaccine has been tested on more than 2,000 volunteers. The company has also recruited the alfalfa looper virus to assist in producing proteins for experimental vaccines to defend people—and poultry—against influenza.

Entomologist Patrick V. Vail discovered the virus in an alfalfa looper caterpillar in 1967. Now at Fresno, California, Vail was at the time working in an ARS laboratory at the University of California, Riverside.

The virus, known as Ac*M*NPV, belongs to a group of microorganisms called baculoviruses. Worldwide, says Vail, scientists have found hundreds of insect-killing baculoviruses.

University and corporate researchers are working to intensify the *A. californica* virus' strength as an insecticide. An example: American Cyanamid, one of this country's largest manufacturers of agricultural chemicals, is intent on building a genetically engineered form of the alfalfa looper virus as a safe, fast-acting bioinsecticide. To accelerate the virus' normally slow rate of kill, company scientists have given it a gene borrowed from the North African brown scorpion, *Androctonus australis*.

Once inside a caterpillar, this upgraded alfalfa looper virus commandeers the insect's own cells so that they carefully follow the scorpion gene instructions, newly encoded in the virus. The directions cue the cells to churn out a new protein—a toxin from the scorpion's venom—that in turn paralyzes the insect.

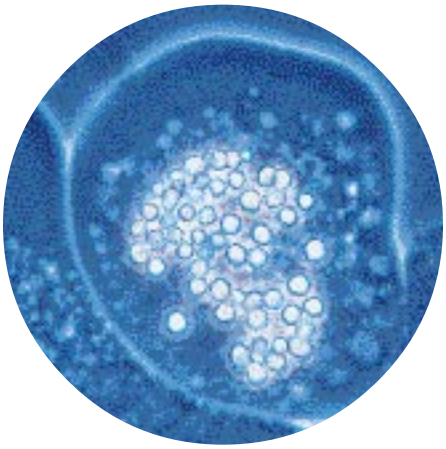
The toxin targets only insects. A pestiferous caterpillar that munches on a leaf with the virus on it soon is unable to chew or crawl. That means the insect can't indulge in its usual feeding frenzy. Normally, a healthy caterpillar can eat many times its weight in food every day as it prepares to pupate—the final life stage before becoming a moth.

The genetically engineered virus takes about 2 to 3 days to kill a caterpillar pest. During that time, the virus replicates inside the hapless insect. Soon the scorpion's paralytic toxin causes the caterpillar to fold up like a little accordion. Later, the insect dies and tumbles from the plant. American Cyanamid scientists estimate that the bioengineered virus may work as much as 60 percent faster than the naturally occurring *A. californica* virus.

The company's tests in the lab and in cottonfields have shown that the virus is harmless to beneficial insects—honey bees, ladybugs, and praying mantids, for example. Similarly, the virus in its natural form or enhanced with the borrowed toxin won't affect people, pets, wildlife, or other organisms—only specific caterpillars. That's according to American Cyanamid's Thomas L. Merriam, director of the team responsible for developing new insecticides.

Besides increasing the virus' speed by pairing it with the insect-specific scorpion toxin, American Cyanamid scientists want to boost production of the virus in fermentation vats.

Traditionally, viruses such as *A. californica* have been produced in laboratory colonies of wiggly insects such as cabbage loopers. As the infection progresses, the virus proliferates. Finally, when they have become severely infected, the loopers are popped into a blender. The new



Left: Dead cabbage looper larva (shown about 3 times actual size) was infected with *AcM*NPV virus. Right: An insect cell nucleus filled with *AcM*NPV polyhedrons (magnified).

supply of virus that was produced in their bodies is then harvested by extracting it from this puree.

However, this method can't be scaled up to yield the copious amounts of genetically engineered virus needed for nationwide marketing. That's because the retooled virus—enhanced with the scorpion toxin gene—works faster than the wild types that exist in nature. An infected insect dies before the virus has a chance to reproduce as profusely as it otherwise would.

Prolific production of the virus is vital, if the bioengineered insecticide is to complement or perhaps even replace some of the synthetic insecticides used today to combat caterpillar pests of cotton and vegetable crops. The hoped-for quantities would be far larger than anything produced today in research labs. The fermenter-based strategy for keeping the virus alive and rapidly reproducing relies on cultures of insect cells, floating in a nutrient-rich liquid.

Instead of multiplying inside captive insects, the virus would live and reproduce in these cultures of disembodied cells of the fall armyworm or other caterpillars. Inside infected cells, the virus' genetic material takes over the cells' functions. The cells read—and follow—the instructions carried in the virus' genome, rather than their own.

In this case, cells are cued to make more copies of the virus genes, complete with the scorpion toxin gene. The newly made virus would then be separated from the insect cells and the culture liquid.

The fermenter process would be somewhat like the method already used today in medical research to produce new proteins. There are, however, two key differences. First, the fermenters would produce the virus in much larger quantities. Second, in biomedical labs, the intent is to have the virus exude the experimental protein into the broth. But in manufacturing a bioinsecticide, the virus itself—with the new toxin gene inside—is harvested.

Scientists at more than 500 laboratories around the globe have exploited the virus to make more than 600 promising proteins. Those include proteins to diagnose or prevent colon cancer, breast cancer, and malaria in humans and bluetongue, rabies, and foot-and-mouth disease in animals. Texas A&M University scientists hold patents for this phenomenally successful use of the virus. Lab supply companies that market the virus in kits offer the product as an alternative to using cells of *Escherichia coli* bacteria or mammalian cells.

Vail, director of the ARS Horticultural Crops Research Laboratory in Fresno since 1987, was recently named the agency's top scientist. And earlier this year, he won an honor award from the U.S. Department of Agriculture. These prizes acknowledge not only his work with the alfalfa looper virus, but also his pioneering studies of other helpful baculoviruses as well.—By **Marcia Wood,** ARS.

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What's in a Name?

The *Autographa californica* virus' full name—"*Autographa californica* multiply embedded nucleopolyhedrosis virus"—not only describes the insect in which the virus was discovered, but also depicts the virus' distinctive packaging.

Commonly abbreviated Ac*M*NPV, the virus occurs in crystalline cases that the alfalfa looper, *A. californica*, or other destructive caterpillars eat. The cases are called polyhedrons because of their many sides.

"Polyhedrons seen through a microscope," says Ac*M*NPV discoverer Patrick V. Vail, "look like little geodesic domes."

The term "nucleopolyhedrosis" refers to these polyhedrons and to the fact that the virus infects the nuclei of insect cells. Each polyhedron typically contains a large number of separate bundles. A bundle encloses within its own membrane one to eight stick-shaped virus particles, or rods. The terms "multiply" and "embedded" refer to the many virus rods packaged in distinct bundles inside a polyhedron.