PEGGY GREB (K9395-1)

Deadly Defenses Could Squelch Mosquitoes

You're strolling along, enjoying the night air, when you're suddenly attacked! Whack! You get the assailant—but other mosquitoes are on their way, seeking your blood as an evening snack.

ARS researchers are using new biological controls to retaliate against the mosquito, an insect that can transmit viruses, protozoan parasites, and filariae (small worms). Such defenses would be useful here in the United States and vital in other countries where mosquito-carried diseases such as malaria are a major health problem.

In the United States, other mosquito-transmitted viruses, such as West Nile virus and St. Louis encephalitis, are making headlines.

West Nile virus, transmitted by the northern house mosquito, *Culex pipiens*, first appeared in the United States in 1999. It was identified in Uganda's West Nile District in 1937. The virus causes brain and spinal cord inflammation in humans. West Nile virus infects many different bird species, but it appears to be lethal to crows, jays, and hawks. Mosquitoes feed on birds infected with the virus and then transmit it to humans and other animals.

St. Louis encephalitis is not new to the United States. It first appeared in St. Louis, Missouri, in 1933, and is the most common mosquito-transmitted disease in the United States. Mild infections cause fever

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Image from an electron microscope of a nucleus in *Culex nigripalpus* infected with a baculovirus. Entomologists Jimmy Becnel (foreground) and Don Barnard examine ultrastructural features of a mosquito baculovirus with an electron microscope.

***Culex nigripalpus* larvae.**

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or headaches. Symptoms of severe infections include headaches, high fever, coma, tremors, convulsions, paralysis, or even death.

A Baculovirus for a Blood Sucker

Entomologist James J. Becnel has found a new baculovirus (a virus specific to arthropods) that kills mosquitoes carrying St. Louis encephalitis and West Nile virus. Becnel, who is with the ARS Center for Medical, Agricultural, and Veterinary Entomology's Mosquito and Fly Research Unit in Gainesville, Florida, discovered the baculovirus in Florida in *Culex nigripalpus* and *C. fasciatus* mosquitoes. The virus kills mosquitoes in the *Culex* genus, which contains more than 500 species.

"No one has looked into using baculoviruses for mosquito control in 25 years. While baculoviruses have been used extensively for controlling *Lepidoptera (*moth-type) species, baculoviruses from mosquitoes were rare, and transmitting them was difficult," Becnel says. "We've been able to get our new baculovirus to kill *Culex nigripalpus* mosquito larvae in 48 to 72 hours. We can infect 60 to 70 percent of mosquitoes in natural populations and 100 percent in the laboratory."

Killer Cocktail

Magnesium is the key to Becnel's mosquito-killing tactics, because it activates the baculovirus. Becnel developed a special cocktail that is added to water where mosquitoes breed. Mosquitoes die when they drink the water containing the virus. The higher the baculovirus concentration in the water, the longer lasting the mosquito control.

"This baculovirus is very specific—it only infects mosquitoes—and doesn't pose a threat to plants or wildlife," notes Becnel.

"Another significant finding is that calcium prevents transmission of the virus to mosquitoes. Understanding how to block as well as transmit the baculovirus is crucial to developing successful control formulations for various habitats. This knowledge may also be applied to other industries that use baculoviruses," Becnel says.

Becnel has filed for patents on his discovery. He says this baculovirus could lead to a new, safe, cost-effective, and specific biological control for mosquitoes that carry St. Louis encephalitis and West Nile virus. Gainesville scientists are testing the effectiveness of the baculovirus on *Culex* mosquitoes carrying West Nile virus in the Northeast.

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Research associate Bettina Moser examines a sample of mosquito baculovirus purified on a gradient.

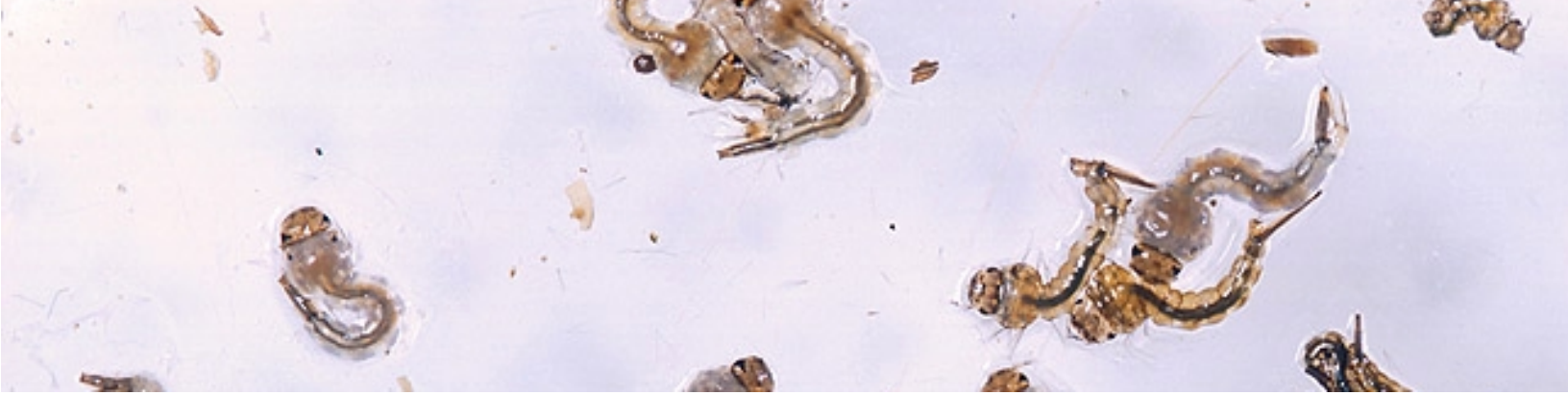
The Gainesville researchers are working with New York colleagues at ARS' Plum Island Animal Disease Center to sequence the entire genome of the baculovirus that infects *Culex* mosquitoes.

"We want to develop a germplasm repository that has genetic information on a large number of mosquito viruses," says Donald R. Barnard, who heads the mosquito unit. "Sequencing these viruses will help us determine which genes are responsible for disease resistance or transmission in specific mosquitoes. This will help us develop and test the baculovirus in other mosquito species."

Controlling an International Pest

ARS researchers are also developing a biocontrol agent for yellowfever mosquitoes, *Aedes aegypti,* which are pests of humans and livestock worldwide. These mosquitoes transmit yellow fever virus and dengue virus, which can cause dengue hemorrhagic fever. They develop in almost any container that collects rainwater, such as barrels, tanks, old tires, cups, cans, and bottles.

The Gainesville experts are working with one of the first biological control agents specific for *A. aegypti* mosquitoes. *Edhazardia aedis* is a naturally occurring protozoan parasite that infects and kills these mosquitoes. A scientist discovered the parasite in Puerto Rico in 1930. It was rediscovered in Thailand in 1979. Becnel acquired regulatory permission to import the parasite into the United States in 1993 for ARS researchers to study. When adult female mosquitoes are infected, tiny spores pass the parasite to their offspring. Most of the larvae die, although some survive to adulthood and spread the infection to other mosquitoes.



In other mosquito species, however, the parasite kills the larvae and is not spread to the next generation. But *A. aegypti* adults will spread the pathogen as they lay eggs and move from one water-filled container to another. Barnard says this is the first pathogen-based biocontrol technology for container-bred mosquitoes.

Federal and state regulatory agencies have already granted ARS a permit to release *E. aedis* in the United States, the first ever for an exotic mosquito pathogen. "We completed field tests in Florida showing *E. aedis* is highly effective against *A. aegypti* mosquitoes. It completely eliminated test mosquito populations in 11 weeks," says Becnel.

Other Nations May Benefit

E. aedis could also help other countries with mosquito problems. Brazilian researchers are especially interested in this biological control. ARS and the Federative Republic of Brazil have established a formal agreement to introduce *E. aedis* in Brazil to control *A. aegypti* mosquitoes.

"Dengue fever and malaria are major diseases transmitted by these mosquitoes in Brazil," says Brazilian entomologist Paulo Vilarinhos, coordinator of Brazil's National Biological Control Program. "Dengue is present in all five Brazilian regions. And we expect the number of cases of mosquito-related diseases to increase as a result of increased agricultural production and more people moving near rivers and forests." These conditions provide more breeding sites and hosts for mosquitoes.

A. aegypti infests 3,650 counties out of Brazil's total 5,500. Brazil started a full-scale eradication program in 1997 using chemical controls. Vilarinhos says, "Although we got more than a 50-percent reduction in dengue cases from 1998, we'd like to reduce or replace chemical use with a biological alternative. The mosquitoes are starting to develop resistance to some chemicals."

About 205,000 dengue cases are reported in Brazil each year. The last dengue epidemic occurred in 1998 and resulted in 540,000 cases. Malaria is a major problem in the country's northern region, with 600,000 cases reported annually.

Worldwide, dengue is one of the most important arthropod-transmitted human viral diseases. About 50 million to 100 million cases of dengue hemorrhagic fever are reported annually. *A. aegypti* mosquitoes are the major dengue vector in urban tropical and subtropical regions of the world.

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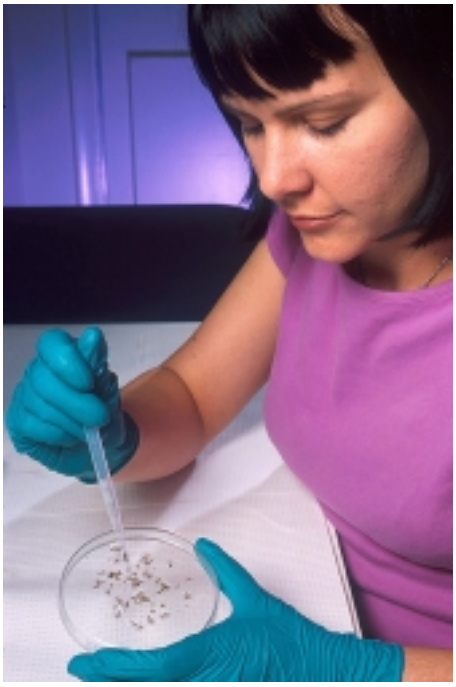

Technician Heather Furlong examines *Culex nigripalpus* larvae for infection with the baculovirus.

No vaccine is available for dengue. Barnard says the only effective prevention method is to control the mosquitoes. He says this new biological control should help do just that while reducing chemical use, environmental pollution, and human and animal pesticide exposure.

ARS scientists are working with Vilarinhos to see whether *E. aedis* can be established in natural mosquito populations in Brazil. "We're very enthusiastic about its potential," says Vilarinhos.

The team will establish test sites in Brazil's five regions, each of which has a different climate. The researchers want to know whether climate will alter *E. aedis'* effectiveness. *E. aedis* will be deployed to the sites in 2001. "We're hoping this biocontrol can collapse the *Aedes* mosquito populations," says Vilarinhos.

"If our tests are successful in Brazil," says Barnard, "it could have an impact on mosquitoes and mosquito-transmitted diseases throughout the world."—By **Tara Weaver-Missick,** ARS.

This research is part of Arthropod Pests of Animals and Humans, an ARS National Program (#104) described on the World Wide Web at http://www.nps.ars.usda.gov.

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