Mauling Mosquitoes Naturally

New ways to silence the buzz

By TINA ADLER

Tell Donald Barnard about children you knew who would run out of their beach houses to watch the bug trucks spray DDT, and he'll one-up you. As a kid living on an army base in Cherry Point, N.C., he "used to go out and run through the [DDT] mist every night," he says.

After discovering that DDT harms wildlife, the federal government outlawed use of the insecticide in the United States, though companies continue to export it legally (SN: 3/16/96, p. 174). Local governments still spray for mosquitoes, but instead of a heated mixture of oil and DDT, they usually employ malathion, an insecticide that degrades rapidly.

Barnard is now one of the many entomologists looking for safer methods of controlling mosquitoes. The scientists want to provide biological alternatives to the chemical insecticides that can damage the environment and endanger people's health. Malathion, for example, kills some beneficial insects and can sicken and even kill humans.

As part of this effort to expand the antimosquito arsenal, researchers are producing a protozoan, a fungus, and an Argentinean nematode that readily multiply. In addition, they have fashioned meals of poisonous bacteria and blue-green algae for mosquitoes to dine on. Some scientists are also developing new repellents, as well as methods of luring the bugs to their deaths.

Entomologists no longer talk of eradicating pests, says Barnard, who heads the mosquito and fly research unit at the U.S. Department of Agriculture's Agricultural Research Service (ARS) in Gainesville, Fla. They know better. They now assume that bugs will become resistant to just about any poison—synthetic or natural—that researchers promote.

Some individual insects in a given population are likely to have a genetic resistance to any insecticide. Those chosen few can survive and reproduce, passing on their good fortune to their offspring, which thrive. Eventually, most of the insects in succeeding generations survive the insecticide.

This resistance problem has increased the need for new mosquito controls. Besides driving even hearty outdoor enthusiasts crazy, the creatures transmit a variety of diseases, such as malaria, dengue, yellow fever, and encephalitis, that kill or sicken millions of people worldwide.

Aedes aegypti mosquitoes carry the viruses that cause dengue and yellow fever. However, a protozoan, *Edhazardia aedis,* infects and kills those mosquitoes naturally, U.S. Army scientists in Thailand found in 1979. So ARS scientists in Gainesville are investigating how to make *E. aedis* a public health servant.

E. aedis produces two versions of insecticidal spores. Mosquito larvae ingest one type, which not only reproduces in the bugs' tissues but also forms another spore type that invades the females' eggs. Some larvae survive the invasion of their tissue, but most larvae that emerge from the contaminated eggs die, and any female larvae that survive produce infected eggs.

Spores also waft from inside dead larvae, mosquitoes ingest them, and the cycle of destruction begins again, explains James J. Becnel of ARS in Gainesville.

Becnel and his colleagues plan to run field tests on *E. aedis* this fall in Argentina, one of the countries where the mosquito remains a serious problem. Because its spores spread so well, the parasite looks like a promising insecticide, he says. "If you can find [an insecticide] that will recycle over time, then you've got something."

A fungus, *Lagenidium giganteum*, also has a supply of mosquito-maiming weapons. Some California farmers have already experimented with this fungus, which survives only in clean, fresh water. The fungus injects deadly spores into larvae, which die 1 to 3 days later. The spores then move to new host mosquitoes, explains Robert K. Washino of the University of California, Davis, who first investigated the insecticidal properties of *L. giganteum*.

In 1992, EPA approved the use of the fungus on soybeans, rice, in grazing pastures, and in any nonagricultural settings, such as wetlands. AgraQuest, a natural pesticide company in Davis, recently began developing techniques for mass-producing the fungus and improving its shelf life. Beginning in July, the company hopes to sell the product in California under the trade name Laginex. Sales in other states should begin in 1997, says AgraQuest's Duane Ewing.

Researchers are finding promising mosquito fighters in various corners of the microbial world. Strains of the bacteria *Bacillus sphaericus (Bs)* and *Bacillus thuringiensis (Bt)* produce proteins that poison a variety of bug species. *Bs* strains kill two types of mosquitoes that dine on them. *Bt* strains destroy beetles and caterpillars, including gypsy moths (SN: 3/19/94, p. 184). One subspecies, *Bt israelensis (Bti),* targets blackflies and a variety of mosquito species.

Bti and *Bs* have served for about 16 years as "useful, safe, and nonpolluting" insecticides, but they break down easily in sunlight, make enzymes that degrade their toxins, sink rapidly in water, and cost a lot to grow, assert Jian-Wei Liu of the National University of Singapore and his colleagues. Researchers thought that the *Bacillus* toxins would be more successful as insecticides if a different bacterial agent hosted them.

"Several laboratories, including our own, have been investigating the use of genetically engineered gram-negative bacteria as alternative hosts" for the *Bacillus* toxins, they report in the March NATURE BIOTECHNOLOGY.

Liu and his coworkers inserted two *Bs* genes into the gram-negative bacterium *Asticcacaulis excentricus*, causing it to produce one of the three *Bs* poisons. Despite its smaller arsenal, the recombinant bacterium is "as toxic or almost as toxic" as the naturally occurring *Bs* strains, they contend. The engineered *A. excentricus* kills the larvae of *Anopheles* mosquitoes, which transmit the malaria parasite. It also destroys *Culex quinquefasciatus*, which carries the virus that causes St. Louis encephalitis and the parasite that causes Bancroftian filariasis, which can lead to the disfiguring disease known as elephantiasis.

Brewing up large batches of the altered *A. excentricus* would cost less than producing *Bs*, which requires expensive, protein-rich media, Liu and his colleagues suggest. *A. excentricus* also tolerates more sunlight than *Bs* and lacks the enzymes that break down the *Bs* toxins.

The new host floats better than *Bs*, because gram-negative bacteria don't form spores, which sink rapidly in water. That has both advantages and disadvantages. *Culex* don't always feed on the surface, so they may not ingest the toxin.

The Singapore researchers' demonstration that transgenic *A. excentricus* efficiently produces a *Bs* toxin is "very welcome indeed," Christopher F. Curtis of the London School of Hygiene and Tropical Medicine asserts in a comment accompanying the NATURE BIOTECHNOLOGY report.

"There is still some way to go, of course, before transformed *A. excentricus* could be used in tropical breeding places in the real world," he warns. For example, the bacterium must still undergo field tests. Since it features only one poison, he also worries about insects rapidly becoming resistant to it.

Using the engineered *A. excentricus* in combination with other biological or synthetic insecticides against the same species may help slow resistance, suggests Alan G. Porter of the National University of Singapore and a coauthor of the report.

The blue-green alga *Agmenellum quadruplicatum* has also become a purveyor of *Bti* poisons. S. Edward Stevens Jr. and Randy Murphy of the University of Memphis genetically engineered the alga to manufacture the toxins. The team is now working to increase the amount of toxin that individual cells of the transgenic alga produce.

Scientists at Cyanotech Corp. in Kailua-Kona, Hawaii, are investigating how to grow large quantities of the souped-up alga, says company president Gerald Cysewski.

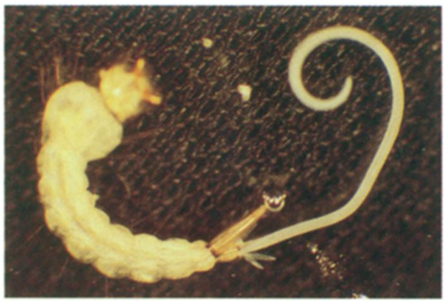
Simply repelling mosquitoes ranks high on the wish list of scientists and outdoor enthusiasts. To do that without smelling like a chemical factory, some consumers have taken to using Avon Products' Skin-So-Soft bath oil, although Avon has never marketed it as a repellent. A company spokeswoman claims the company has not investigated why it appears to ward off insects.

William S. Bowers of the University of Arizona in Tucson has. In recent, unpublished research, he discovered that the product has four ingredients that mosquitoes avoid. One of the strongest, coumarin, smells like new-mown hay and is widely used in perfumes. The Food and Drug Administration prohibits the use of coumarin in food because animal tests show it damages the liver and other organs when ingested.

Coumarin appears to be as effective as the popular chemical repellent DEET (N,N-diethyl-meta-toluamide), Bowers says. But the small amount of coumarin that he found in the bath oil wouldn't scare any bug.

Coumarin shows up in hundreds of plants, including *Ageratum*, a flowering plant that most pests avoid. When Bowers and his colleagues cut an ageratum's leaves in a way that mimics insect damage, the plant released more coumarin than usual. The amount went up again when they rubbed grasshopper saliva in the wounds.

Before heading into bug territory, Bowers dabs himself with coumarin that he buys from science supply companies. He doesn't use ageratum as a personal insect deterrent, because it has some slightly toxic compounds that he'd rather avoid.



Tokuo Fukuda/ARS

A natural enemy of many mosquito species, the Argentinean nematode, Strelkovimermis spiculatus, *matures inside a mosquito larva, then busts out as an adult, killing its host in the process. Here, it is escaping from* Culex quinquefasciatus. *The nematodes can survive in polluted water, where mosquitoes often live. Scientists have raised them in large quantities and hope to test them in the field this summer.*

Despite his appreciation for coumarin, he has no faith in its ever going commercial. Manufacturers have shown little interest in any new repellents because of the high cost of getting them to market. They ignore even synthetic chemicals developed by ARS that rebuff mosquitoes and other blood-sucking insects better than DEET, Barnard says.

DEET provides tough competition. First produced in 1954 by USDA, it is already tested, approved, marketed, and selling well. Each year, consumers buy about $100 million worth of products that contain the chemical.

As eagerly as some scientists are trying to repel mosquitoes, others are working to attract them (SN: 4/18/92, p. 255). But they too have the bugs' demise in mind—they plan to lure them to their deaths.

On Key Island, in the heart of the Florida Everglades' mosquito country, Daniel L. Kline and his colleagues at ARS in Gainesville set up 52 traps that release carbon dioxide, a known mosquito attractant. They also scented the traps with octenol, which has the ever-enchanting aroma of cow's breath. Ruminants breathe out the chemical when they digest grass, and mosquitoes follow the smell to their required meal of mammalian blood.

The Florida scientists laced all their traps with a synthetic pyrethroid insecticide, which kills insects on contact. Since they were set up in 1993, the traps have destroyed billions of salt marsh mosquitoes (*Aedes taeniorhynchus*), eliminating about 90 percent of the population.

While researchers are working out sophisticated methods of controlling pests, some simple actions have remained surprisingly effective, such as retreating into net-covered beds at night, when mosquitoes that transmit malaria are most likely to bite.

Covering beds, windows, and doors with mosquito nets treated with pyrethroid insecticide reduced malaria deaths among children under age 5 by one-sixth in Ghana and by one-third in Kenya during a 2-year observation period, according to two reports in the April TROPICAL MEDICINE AND INTERNATIONAL HEALTH.

In Ghana, where malaria is more prevalent, the study included about 20,000 children, reports Fred N. Binka of the Navrongo Health Research Center. The Kenya study involved about 10,000, says Christopher G. Nevill of the Medical Research Foundation in Nairobi. The authors predict that the widespread use of treated nets in Africa could save the lives of about 500,000 children each year.

Another study found that among approximately 5,500 Tanzanian children under age 5, the incidence of malaria dropped from 85 percent to 39 percent in villages that had used the nets for 2 years and to 25 percent in villages that had done so for 3 years, says Clive Shiff of the Johns Hopkins University School of Hygiene and Public Health in Baltimore.

Zulfiqarali Premji of the Muhimbili Medical Centre in Dar es Salaam, Tanzania, Shiff, and their colleagues reported these findings in the October 1995 TROPICAL MEDICINE AND PARASITOLOGY.

Simply closing down local mosquito baths helps too, Barnard points out. Most mosquito problems result from human activities that cause water to pool—an irrigation ditch fills up, a construction site develops puddles, even upside-down garbage lids and cups left outside serve as nurseries for the critters.

So the natural approach to reducing mosquito problems is advancing on two fronts. Some researchers are engineering and mass-producing sophisticated biological weapons. Other insect foes are focusing their efforts on simply keeping people out of the reach of the mosquito's sting. □