An Inside Job

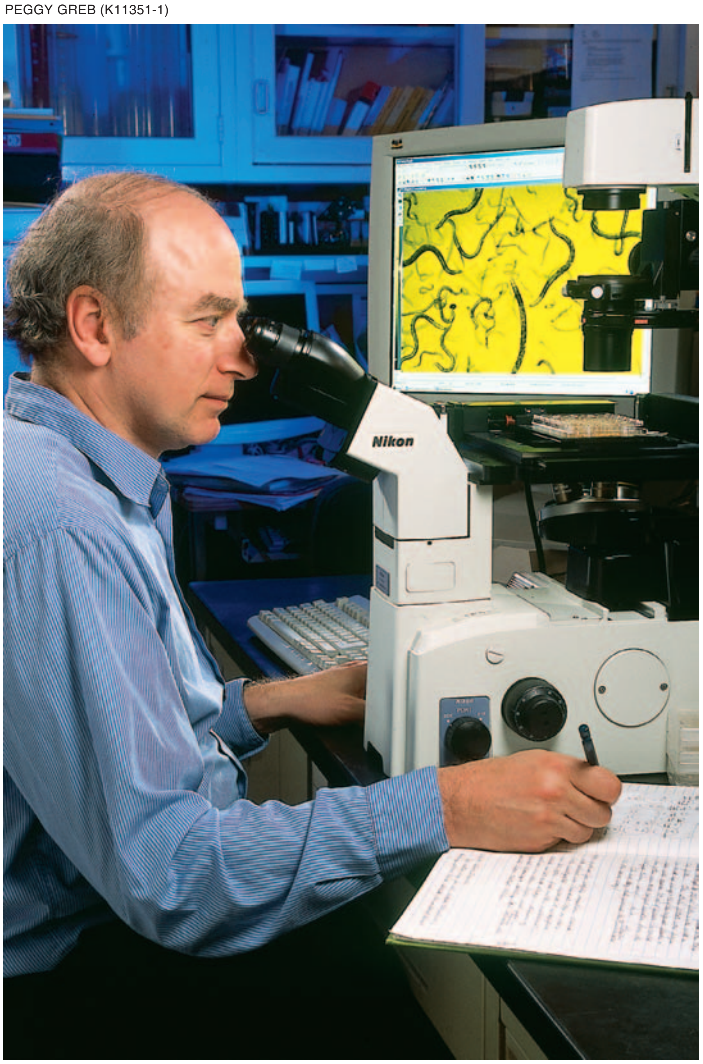
Using nematodes' own biology against them

ARS scientists are looking within for environmentally friendly ways to battle nematodes. Within the nematode, that is.

"We want to find out what makes nematodes tick, and what we can do to them to make them stop ticking," says zoologist David Chitwood. He's research leader of ARS's Nematology Laboratory in Beltsville, Maryland.

These microscopic plant-parasitic worms cost U.S. farmers more than $10 billion in losses annually. Chitwood's team is probing nematodes' genes and proteins, susceptibility to toxins, and even their cholesterol levels for vulnerabilities. "We're examining basic processes like locomotion, egg hatch, and growth and development," says physiologist Edward Masler. "We're focusing on 'housekeeping molecules' that help maintain cell health and structure."

PEGGY GREB (K11351-1)



Zoologist David Chitwood examines the effects of an inhibitor of sterol metabolism on the nematode *Caenorhabditis elegans*. Inhibiting the sterol metabolism of nematodes may be one way of turning their biology against them.

Masler and molecular biologist Andrea Skantar studied whether heat-shock proteins (HSPs) are soft spots in nematodes' armor. As a result, Skantar made the first-ever report of an important HSP in soybean cyst nematodes, the world's most important soybean pest. "HSP-90 regulates specific target proteins that control normal cell development and metabolism," she says. "It also appears to govern adaptation to environmental extremes, such as starvation and temperature stress, in many organisms."

Testing a Natural Product

Skantar and plant pathologists Lynn Carta and Susan Meyer were able to inhibit the action of HSP-90 in free-living *Caenorhabditis elegans* and plant-parasitic *Heterodera glycines* nematodes. They used geldanamycin, a compound produced by bacteria.

"We exposed nematode eggs to geldanamycin in multi-well petri dishes and discovered that it reduced hatching and hampered juveniles' ability to move about," she says. "This is important because these early life stages, which take place in soil, are vulnerable to attack with biologically based measures."

The next goal is to determine whether the geldanamycin-producing bacteria have potential as a biocontrol agent against nematodes. Although these common bacteria have stymied fungi and other plant pests, Skantar's upcoming tests will mark the first time they will be evaluated against nematodes.

Actin Gene an Enticing Lead

Masler's work led to the discovery of HSP-70, a protein that helps plant-parasitic cyst nematodes respond to stress. It also led to the first description of the actin gene in these nematodes. Actin is an abundant cellular protein active in muscular contraction, cellular movement, and cell-shape maintenance in most organisms.

"Actin is essential for nematode development, survival, feeding, and reproduction," says Masler. His group cloned and characterized the gene from the soybean cyst nematode *H. glycines;* a potato cyst nematode, *Globodera rostochiensis;* and the free-living (nonparasitic) *Panagrellus redivivus*.

"We're interested in actin because it interacts closely with other cell molecules and can lead us to those that are unique to nematodes," says Masler. "By focusing on such molecules, we can design control agents specific to nematodes but harmless to other organisms."

Meanwhile, Carta and colleagues led by University of California-San Diego professor Raffi Aroian have evaluated the nematode-killing properties of toxins found in *Bacillus thuringiensis (Bt),* a safe, common bacterium. Some *Bt* strains are currently used against insects that attack crops and carry human diseases. The active agents for pest control are called "crystal toxins," and they target the gut.

The team cloned seven of *Bt's* nematode-specific crystal proteins into *E. coli* bacteria and tested them against five bacteria-feeding nematodes and one that parasitizes animals.

"Four toxins damaged the gut of at least two nematode species," says Carta. "Each of the six nematode species was damaged by at least one toxin. This is the first report on the killing of nematodes in general by *Bt* crystal toxins independent of *Bt* bacteria. It shows how well these different toxins damage various nematodes."

Carta adds that it's also the first report of crystal toxins harming an animal parasite. "This provides a foundation for future use of these mammalian-safe toxins against plant-parasitic nematodes that Dr. Aroian's laboratory is currently investigating," she says.

PEGGY GREB (K11361-1)

Physiologist Edward Masler observes as technician Carol Robinson treats soybean plants with soybean cyst nematode eggs. The eggs hatch and juvenile nematodes then enter the soybean roots where they mature and reproduce. Large numbers of new nematodes are harvested and used for gene analysis.

Focus on Sterols

Chitwood is studying ways to disrupt biochemical development pathways in nematodes. He's focusing on the pathway for sterols—chemical compounds found in the cells of plants and animals. The most common of these is cholesterol.

"Nematodes can't make their own cholesterol," says Chitwood. "But they can use nonfunctional sterols found in their hosts and convert them into cholesterol and other sterols for cellular function."

Previous studies in *C. elegans* found that blocking sterol conversion to cholesterol through use of azacoprostane causes serious defects in germ cell and cuticle development, growth, and motility. It also specifically inhibits at least one enzyme involved in nematode sterol metabolism. Azacoprostane is a chemical analog of cholesterol developed by ARS chemists during the 1980s.

In a followup, Chitwood, along with researchers Byung-Kwon Choi and Young-Ki Paik from Yonsei University in Seoul, South Korea, sought to learn exactly how disruption of sterol metabolism actually kills nematodes. They focused specifically on the effects of azacoprostane on nematodes' synthesis of different kinds of proteins.

"The results indicated that treated nematodes contain low levels of some important proteins usually abundant in their body walls and other proteins involved in transporting fats within them," says Chitwood. "They showed that nematode sterols regulate synthesis of their proteins."

Chitwood says all these studies may lead to safe, innovative strategies for managing this important crop pest. "We're turning the nematode's own biology against itself," he says. "This can be put into practical use by applying compounds to soil that re-create our results or by engineering plants to produce antinematode compounds or genetically based resistance."—By **Luis Pons,** ARS.

This research is part of Plant Diseases (#303) and Methyl Bromide Alternatives (#308), two ARS National Programs described on the World Wide Web at www.nps.ars.usda.gov.

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PEGGY GREB (K11357-1)

Molecular biologist Andrea Skantar uses a gel imaging system to analyze HSP-90 genes obtained from different soybean cyst nematode populations. Variations in HSP-90 may relate to nematode stress responses or sensitivity to geldanamycin.

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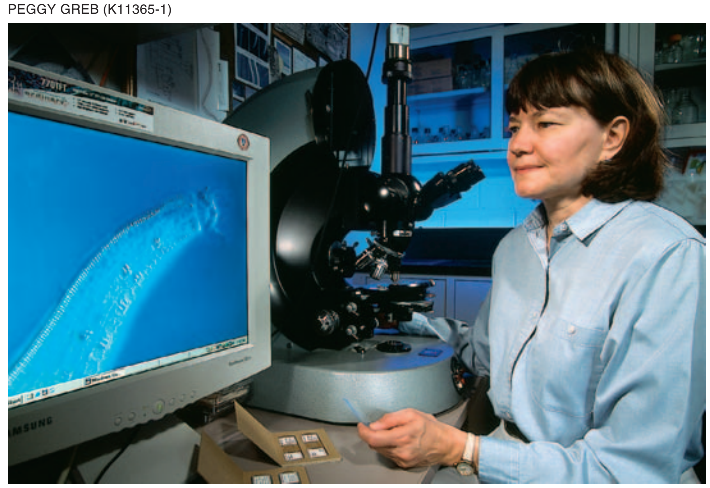

Using a high-powered compound microscope, plant pathologist Lynn Carta examines the head and neck of a nematode. Related nematodes ingested bacteria containing *Bt* toxins that killed them. Nematode-specific toxins like *Bt* may be used as a future biocontrol.