# A Soil Story

## Life inside a nematode: Creative chemistry and novel pest control

By CHRISTINE MLOT

There are 8 million stories in the Naked City—and at least that many in a spadeful of dirt.

This is one of those stories, a tale of microbes and microscopic worms that terrorize maggots, caterpillars, and other insects that dwell in the soil.

Although hints of the story appeared in the last century, when biologists came across dead insects laced with tiny roundworms, or nematodes, the full details are still being worked out. By the 1970s, microbes that live symbiotically in the gut of the nematodes had been identified as the real insect killers. The relatively few researchers who have since looked at the chemistry of these bacteria have been astonished at what they've found.

"They're extremely weird," says environmental microbiologist Kenneth H. Nealson of the University of Wisconsin-Milwaukee's Center for Great Lakes Studies. Another researcher calls the colorful microbes "luscious." At the May meeting of the American Society for Microbiology in Miami Beach, Nealson and his colleagues described their work on one aspect of the bacteria's unusual biochemistry—bright red pigments that behave like antibiotics.

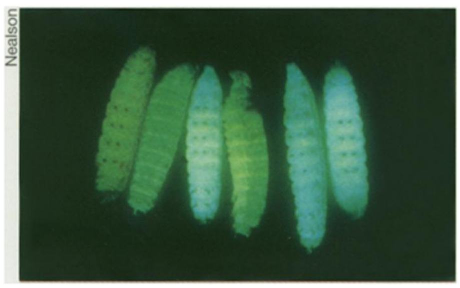
Besides the pigments, the bacteria churn out other antibiotics and huge amounts of an intriguing crystalline protein. In addition, the bacteria's insect-killing toxin can take out a wide range of pests and has recently gone into development for potential commercial use.

The bacteria also pull off perhaps the most alluring of feats in biochemistry: They make light.

"They really do it all," says microbiologist David J. Bowen of the University of Wisconsin-Madison.

George O. Poinar Jr. of Oregon State University in Corvallis has puzzled out much of the insect-microbe-nematode triangle. In the animal world, nematode species are second in abundance only to insects, but just a few kinds are known to carry the lethal microbial weapons.

It all happens in the dark interstices of a clump of earth, in the wild or in a backyard. Juvenile *Heterorhabditis* nematodes, transparent and a millimeter long, cruise for insect prey. Barely visible to the human eye, one of these hair-thin creatures can take on a caterpillar many times its size.




*Dead caterpillars glow from the light produced by bacteria that have streamed through their circulatory system.*

When it comes across an insect larva, the nematode slips in through the mouth or another opening. It bores a hole into the insect's circulatory system and enters it, but the real devastation comes when the nematode expels the bacteria from its intestine.

These bacteria stream through the insect, setting up "a raging septicemia," says Jerald C. Ensign, a microbiologist at the University of Wisconsin-Madison. In about 24 hours, the insect is dead.

For the other two organisms of the triad, it's a feast day.

"The bacteria digest the insect and grow like gangbusters," says Nealson. They also begin to shine—literally. Fittingly, the bacteria were recently renamed *Photorhabdus luminescens.*

Like certain marine bacteria (SN: 9/14/96, p. 167), *P. luminescens* contains the genes for luciferase, the enzyme that trips the switch of a light-emitting biological reaction. When the reaction runs, the insect cadaver, filled with the luminous bacteria, casts an eerie, funereal glow. At other times, the bacteria produce pigments that color the caterpillars brick red.

Meanwhile, the nematode is completing its life cycle by sexually maturing into a hermaphrodite and reproducing. "A single nematode will do the job," says Nealson. It fertilizes its own eggs and lays them inside the insect corpse. In a macabre ménage à trois, the newly hatched nematodes feed on the bacteria feeding on the dead insect.

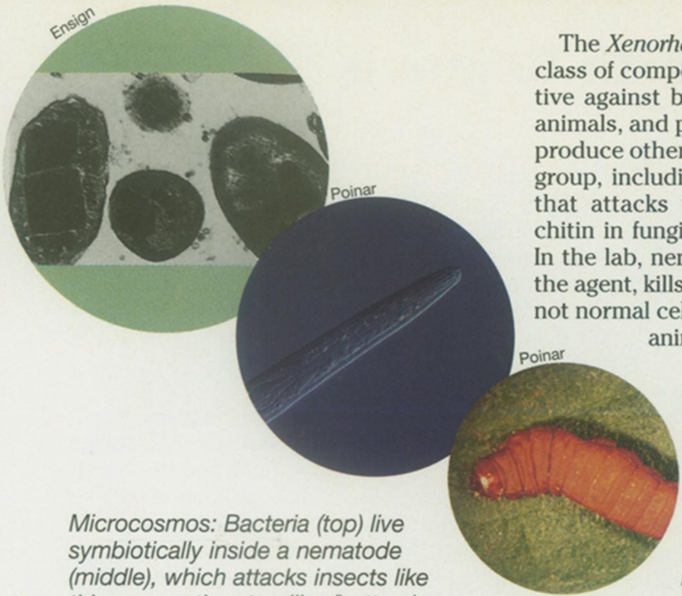
After 10 to 14 days, perhaps when nutrients from the insect start to run dry, the nematodes stop ingesting the bacteria as food and instead take them in as intestinal symbionts. This shift is one of the yet-to-be-explained aspects of the association. After a couple of cycles of egg laying, thousands of juvenile nematodes packed with *P. luminescens* emerge from the remains of the insect and head back into the underground.

Why would bacteria that live in a nematode's intestine be genetically equipped to produce biological luminescence? "My guess would be that it has something to do with distribution," says Nealson. The light might attract other insects for a future kill. Or it may be a beacon to a passing bird, which could pluck up the glowing or bright red morsel and deposit it in new territory.

Poinar says the luminescence may simply be an accident of evolutionary history. Since this bacterium is the only terrestrial microbe known to luminesce, it may have evolved from a marine species, perhaps when a nematode crawling along a beach swallowed some of the luminous organisms. In the new environment, the selective force for the genetic lighting equipment may not exist and the light, an energetically expensive phenomenon, may be fading.

In a pitch-black incubation room, it takes a few moments for the human eye to notice the white-blue glow radiating from flasks where cultures of the bacteria are kept.

"It's a real subtle luminescence," says Nealson. "It doesn't knock you out like the marine ones." In an article in the March 1996 MICROBIOLOGICAL REVIEWS, Nealson suggests that *Photorhabdus*, which does not seem to be closely related to the lumi-




*Microcosmos: Bacteria (top) live symbiotically inside a nematode (middle), which attacks insects like this wax moth caterpillar (bottom). The oblong bacteria are filled with a crystalline protein. They also produce a toxin that kills insects and make pigments that can turn the insects brick red.*

nous marine bacteria but does share much of the same genetic sequence for luciferase, acquired the gene from another bacterial species, perhaps via a virus or some other mechanism.

It's easier to explain the function of the microbial antibiotics. Once killed, the insect is a ripe target for fungi and other microbial decay specialists. The bacteria make a variety of unusual broad-spectrum antibiotics, probably to keep the competition down.

Nealson cites reports by military doctors early in the last century that tell of the "phosphorescence of wounds." This was taken as a sign of healing and may well have been the work of *Photorhabdus* bacteria producing antibiotics that combated nastier infections.

Nealson has found that the pigments, known as anthraquinones, have the same basic structure as erythromycin and similar antibiotics produced by more familiar bacteria, even though *Photorhabdus* is in an unrelated family not known to produce such antibiotics.

The luciferase, antibiotics, and other chemical tricks of the bacterium seem to have come from a variety of unrelated bacteria. Says Nealson, it's as if *Photorhabdus* "stole a whole bunch of information from somewhere. Phylogenetically, they shouldn't be doing what they're doing."

John M. Webster of Simon Fraser University in Burnaby, British Columbia, studies the antibiotics produced by the closely related *Xenorhabdus* bacteria, which live symbiotically within *Steinernema* nematodes. These bacteria produce pigments and antibiotics that are very different at a molecular level from those of *Photorhabdus,* even though other properties of the two bacteria are so similar that until recently they were thought to be the same organism.

The *Xenorhabdus* antibiotics are a new class of compounds, says Webster, effective against bacteria that attack plants, animals, and people. These bacteria also produce other chemicals unusual for the group, including a chitinase, an enzyme that attacks the structural compound chitin in fungi, and an anticancer agent. In the lab, nematophin, as Webster calls the agent, kills off human cancer cells but not normal cells; it is now being tested in animals.

The antibiotics were what originally interested Ensign in the nematode symbionts. When he got his first glimpse of *Photorhabdus* through a microscope, however, he switched research gears. Staring back at him from each of the rod-shaped bacterial cells were giant spots of bright crystals. Ensign was familiar with such crystals from another soil bacterium, *Bacillus thuringiensis*, best known for its production of the insecticide Bt (SN: 9/12/92, p. 166; 7/9/88, p. 27).

Yet when he and his coworkers fed or injected the crystals into a variety of insects, nothing happened. The crystals clearly were not responsible for killing the insects.

The crystals were essential to the symbiosis, however. When the bacteria were mutated to eliminate crystal production, their nematode partners could no longer thrive.

Analysis of the crystals revealed a protein rich in methionine and lysine, two of the essential amino acids that are sometimes difficult for animals to obtain. That's why lysine, for example, is typically added to animal feed and why corn has been bred to boost its lysine content.

Says Ensign, "there's no other single protein with as much methionine and lysine," and *Photorhabdus* serves up generous amounts of it. The crystals make up about 50 percent of the cell's protein. With its essential amino acids, this crystalline protein is probably a large part of why the nematode hangs on to the bacteria. Ensign says the nutritious protein might be useful as an additive in animal feed or engineered into a food plant.

What, then, kills the insects? Ensign and Bowen turned to purifying and characterizing other proteins produced by the bacteria. They came up with a powerful toxin that is a complex of several proteins. Very small amounts—nanogram quantities—can be fatal to a variety of pesky insects, says Ensign, including the tobacco hornworm (belonging to the order Lepidoptera) and the mealworm (Coleoptera). The researchers say it even kills cockroaches (Dictyoptera) and ants (Hymenoptera).

Other researchers seemed to doubt that the toxin by itself could work as a control agent. It appeared to be effective only when the nematode carried it into the insect's circulation. In contrast, Bt could dissolve a hole in the insect's gut.

Ensign's group has now found that a concentrated dose of their toxin works if a susceptible insect simply eats it. They haven't figured out how it acts, although it seems to cause a severe intestinal upset—an insect stops feeding soon after it gets a dose and is dead after a day or two.

Ensign's collaborators at DowElanco in Indianapolis have isolated the genes for the toxin, and in May, the group completed its patent application. Although the toxin is not yet ready for any large-scale agricultural action, Ensign is optimistic.

With reports in recent years of emerging pest resistance to Bt, "we hope this will be the next generation of microbial insecticides." He envisions that, as with Bt, the genes could be engineered into crop plants so that they produce their own insect-killing toxin, reducing the need for other pesticides.

Nealson says that "it's potentially a really great thing," although there is the possibility that the large toxin may be too complex, with too many genes involved, to be successfully expressed by a plant.

Others have simply put the whole worm to work. Researchers at the Department of Agriculture, for example, have used a *Steinernema* nematode as the pest-seeking carrier of *Xenorhabdus,* which produces yet another toxin.

On the market for about 3 years, this biological complex is being used successfully on citrus weevils in Florida and the grubs of turf pests in the Southeast, according to Agricultural Research Service researcher Jimmy R. Raulston in Weslaco, Texas. The nematodes are sold in a semidehydrated state. They can then be added to water and sprayed on fields or deployed in a greenhouse.

Apart from its commercial prospects, the bacteria-nematode symbiosis is a readily available tool for teaching about an unusual evolutionary niche and some remarkable chemistry, adds Nealson. To catch the nematodes, researchers simply set out caterpillars as bait in buckets of dirt—of any garden variety—then do a surveillance in the dark. Usually, the killer combo marks its victims with its telltale glow.

It may be the only story in the soil that comes with a spotlight—or it may not be.

Recent use of molecular techniques to find and quantify the extent of life in the soil has revealed a staggering number and diversity of microorganisms. Only 5,000 or so species of bacteria have been officially recognized. Yet a single pinch of dirt easily contains that many. The identities of most of those species are unknown, not to mention their stories.

This has been only one of them. □