VIEWPOINT

Entomopathogenic Nematodes for the Biological Control of Insects[1]

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Nematode parasites of insects have been known since the 17th century (33), but it was only in the 1930s, that serious consideration was given to using a nematode to control an insect. In 1929, Glaser and Fox (19) found a nematode infecting grubs of the Japanese beetle, *Popillia japonica*, at the Tavistock Golf Course near Haddonfield, New Jersey. Steiner (44) described the nematode that same year as *Neoaplectana* (= *Steinernema*) *glaseri*. We now know that these nematodes carry a symbiotic bacterium which provides essential food for the nematodes whether they are infecting a host or in culture. Glaser was not aware of the symbiotic bacterium (5); nonetheless, the methods that he devised to culture the nematode in vitro were suitable for the bacterium to multiple (16,17).

Glaser and colleagues produced sufficient numbers of the nematode for field trials and in the 1930s applied it to 73 different field plots in New Jersey to control the Japanese beetle (17,20). Parasitized grubs were recovered from 72 of the 73 plots two weeks after application. Parasitism of the grub population by the nema-

tode in the various plots ranged from 0.3% to 81%. They determined nematode persistence in four of the plots by placing healthy beetle larvae in the plots and later examining them for infection. The nematode persisted in the plots for the 8.5 years of their trials (18,20).

Glaser's outstanding research should have issued in an era of biological control using entomopathogenic nematodes. Instead, the widespread use of the highly effective and relatively inexpensive persistent pesticides during the 1940–1960 period caused Glaser's work to be forgotten temporarily. In the 1960s and 70s, when some persistent pesticides became unavailable due to action by the U.S. Environmental Protection Agency, there was renewed interest in entomopathogenic nematodes as biological control organisms, and investigations on them have been carried out since.

Classification and biology: The genus *Steinernema* is in the family Steinernematidae (Rhabditida: Nematoda). The family contains one other genus, *Neosteinernema*, with a single species, *N. longicurvicauda*, a parasite of termites (31). In 1976, Poinar (38) described a new genus and species of entomopathogenic nematode, *Heterorhabditis bacteriophora*, which he placed in a new family, Heterorhabditidae (Rhabditida: Nematoda). The two genera *Steinernema* and *Heterorhabditis* contain the most important species of entomopathogenic nema-

todes. Currently, there are 16 species in *Steinernema* and six species in *Heterorhabditis.*

All members of the Order Rhabditida are bacteriophagous, and many of them have phoretic associations with insects. Over time, apparently some of the nematodes evolved into insect pathogens. In the Steinernematidae and Heterorhabditidae, an area of the anterior part of the intestine of the infective juvenile is modified as a bacterial chamber. In this chamber the infective juvenile carries cells of a symbiotic bacterium. The bacterium carried by Steinernematidae is usually a species of the genus *Xenorhabdus,* and that carried by Heterorhabditidae is a species of *Photorhabdus.*

Pathogenicity and life cycle: The infective juvenile enters the insect host through the mouth, anus, spiracles, or by direct penetration through the cuticle. If the mode of entry is by mouth or anus, the nematode penetrates the gut wall to reach the hemocoel, and if by spiracles, it penetrates the tracheal wall. When the infective juvenile reaches the hemocoel of a host, it releases the bacteria, which multiply rapidly in the hemolymph. Usually the insect dies within 24–72 hours. Even though the bacterium is primarily responsible for the mortality of most insect hosts, the nematode also produces a toxin that is lethal to the insect (4). The infective juvenile becomes a feeding third-stage juvenile, feeds on the bacteria and their metabolic byproducts, and molts to the fourth stage and then to males and females of the first generation. After mating, the females lay eggs that hatch as first-stage juveniles that molt successively to second-, third-, and fourth-stage juveniles and then to males and females of the second generation. The adults mate and the eggs produced by these second-generation females hatch as first-stage juveniles that molt to the second stage. The late second-stage juveniles cease feeding, incorporate a pellet of bacteria in the bacterial chamber, and molt to the third stage (infective juvenile), retaining the cuticle of the second stage as a sheath, and leave the cadaver in search of new hosts. In some hosts, the second generation is omitted and the eggs that are laid by first-generation adult females develop into infective juveniles. The cycle from entry of infective juveniles into a host from emergence of infective juveniles from a host is temperature-dependent and varies somewhat for different species and strains. However, it takes about 7–10 days at 25 C in *Galleria mellonella* (30,47,48). Differences for the Heterorhabditidae are that all juveniles of the first generation become hermaphrodites. In the second generation, males, females, and hermaphrodites develop.

Dispersal of juveniles: The juveniles of steinernematids and heterorhabditids disperse vertically and horizontally, both actively and passively (8,24,29,35,45). Passively, they may be dispersed by rain, wind, soil, humans, or insects. Active dispersal may be measured in centimeters, while passive dispersal by insects may be measured in kilometers (43).

Survival of juveniles: The infective juveniles do not feed but can live for weeks on stored reserves as active juveniles, and for months by entering a near-anhydrobiotic state. This is almost certainly the most important survival strategy for the nematode. The length of time that juveniles survive in the soil in the absence of a host depends upon such factors as temperature, humidity, natural enemies, and soil type. Generally, survival is measured in weeks to months, and is better in a sandy soil or sandy-loam soil at low moisture and with temperatures from about 15–25 C than in clay soils and lower or higher temperatures (1,24,26–28). The Heterorhabditidae do not survive as well as do Steinernematidae (27).

Natural enemies: Natural enemies play an important role in the population ecology of all organisms. Populations of entomopathogenic nematodes in the soil are reduced by bacteria, fungi, mites, predatory nematodes, tardigrades, and other soil organisms. Survival is better in sterilized soil than in nonsterilized soil (24,29). Mites appear to be especially voracious nematode-feeders (8,28,46).

Insects controlled: Insects controlled with entomopathogenic nematodes have been reviewed by Georgis and Manweiler (15), Kaya (23), Klein (25), by several authors in Nickle (32), and by Wouts (49). Some of the insects controlled are armyworms, carpenter worms, cat fleas, crown borers, cutworms, filth flies, flea beetles, German cockroaches, leaf miners, mole crickets, phorid flies, plume moths, root weevils, sciarid flies, stem borers, webworms, and white grubs.

Advantages of entomopathogenic nematodes: Entomopathogenic nematodes have certain advantages over chemicals as control agents. Nematodes are non-polluting and thus environmentally safe and acceptable, although some countries do not allow the release of non-indigenous species. Infective juveniles can be applied with conventional equipment (13), and they are compatible with most pesticides (6,9,40,41). They find their hosts either actively or passively, and in cryptic habitats and sometimes in soil, they have proven superior to chemicals in controlling the target insect (12). They are not well-suited for foliar application, however, since they are sensitive to desiccation and ultraviolet radiation. The nematodes usually reproduce in the insect host and thus provide new infective juveniles to search for additional host insects. The effective host range of a given species or strain is usually rather narrow, thus they do not cause indiscriminate mortality. The narrow host range means that one must select the appropriate nematode just as one must select the appropriate chemical insecticide to control the target insect.

Rearing: Steinernematid and heterorhabditid nematodes can be reared in vivo in insect hosts or they can be mass-produced in vitro on solid medium or in liquid medium (2,3,7,10,13,21,22). For solid medium culture, a substrate such as beef or pork kidney or liver, or chicken offal may be used. The substrate usually is made into a paste that is coated onto a porous substrate such as sponge. The medium is sterilized, inoculated with the bacterium, and nematodes are added 24 hours later. Infective juveniles are harvested after about 15 days. This method is labor-intensive and is particularly well-suited for situations where labor is plentiful, and for the so-called cottage industry.

Production in liquid medium can be done in small containers or in fermentation tanks. Greater numbers of juveniles can be produced per unit area in fermentation tanks, which makes this method especially suited for large-scale commercial production (10,11).

Comparative costs: In 1991, it was estimated that it costs 10–60% more to control insects with nematode-based products than with chemical insecticides (14). That is changing, however, as technological improvements in production, formulation, packaging, and shelf life of nematode products occur. Since nematode products are safe to apply and do not contaminate the environment, some clients will opt for a biological control method even at a higher cost. Also, at least in some situations, the nematodes become established, recycle, and their offspring continue to control the target insect (35–37). Thus, the higher short-term cost may be lower in the long run when continued control by the recycling nematode is obtained. In Florida, a recreational turf area supervisor, who used *Steinernema scapterisci* to control mole crickets, *Scapteriscus* spp., was surprised at how quickly mole cricket populations were reduced and the turf improved. He was also pleased that he was experiencing continued mole cricket control by the recycling nematodes (pers. comm.). In another case in Florida, the superintendent of a golf course reported (pers. comm.) that he has reduced his chemical budget for mole cricket control by 50% by using *S. scapterisci* and then spot treating as needed instead of using broadcast applications of chemicals as he did previously. Thus, by the judicious use of nematodes and chemicals, it may be possible to reduce the cost of control and protect the environment at the same time.

An excellent example of a situation in

which a nematode may replace chemicals for control of an insect is the black vine weevil, *Otiorhynchus sulcatus,* in cranberries. Uses of chemical insecticides on cranberry either are restricted or have not provided adequate control of black vine weevil larvae. *Heterorhabditis bacteriophora* NC strain was applied, and it provided more than 70% control soon after treatment and was still providing that same level of control a year later (42).

Commercial products: Most of the nematode-based products currently available are formulations of various strains of *Steinernema carpocapsae* such as ORTHO Bio-Safe, BioVector, and Exhibit in the United States, Sanoplant in Switzerland, Boden-Nützlinge in Germany, and Helix in Canada. Other species of *Steinernema* commercially available are *S. feltiae* as Magnet in the United States, and as Nemasys and Stealth in the United Kingdom, *S. riobravis* as Vector MC and *S. scapterisci* as Proactant Ss in the United States. *Heterorhabditis bacteriophora* is available as Otinem in the United States and *H. megidis* as Nemasys in the United Kingdom. There are "cottage industry" companies that sell nematode products, most of which contain strains of *S. carpocapsae.*

Future role: The future of nematode-based products for insect control is excellent. The technology used currently for producing, formulating, packaging, storing, and shipping nematode products was developed during the past 15 years, even though some of the technology is more than 60 years old. Since the first commercial products were developed, vast technological improvements have been made. Future improvements may well make today's technology obsolete. More efficient methods of production, formulation, etc. will lower the cost of nematode products and make them more competitive economically.

Even though a total of 22 species of the two genera of entomopathogenic nematodes have been described, only six have been commercialized: *S. carpocapsae, S. feltiae, S. riobravis, S. scapterisci, H. bacterio-* *phora,* and *H. megidis.* Some of the described species were discovered in the last few years as more scientists became interested in entomopathogenic nematodes. There is every reason to believe that additional species which are pathogens of pest insects not now targeted will be discovered in the future. These species, and perhaps some currently described, will add to the arsenal of nematode weapons aimed at pest insects.

"Brighteners" which protect nematodes from harmful ultraviolet radiation (34) and antidesiccants may well be combined in the future to formulate nematode products to be used as sprays to control foliar insects. Such technology would open up vast markets.

When one considers that the sale of commercial products, except by small producers for local markets, is no more than 15 years old and in the United States only a little more than 5 years old, the potential market has not begun to be realized. While *s. carpocapsae* was the first nematode product marketed, *S. scapterisci* became available commercially only in 1993, and *S. riobravis* in 1994.

Currently, the share of the pest control market captured by entomopathogenic nematodes is probably less than 1%. The share likely will remain relatively low for the foreseeable future, but will increase due to more efficient production methods and the demands of the public for safer, more environmentally acceptable products. As the user fully realizes that nematodes are biological organisms and must be handled as such to provide effective control, greater acceptance of nematode-based products will occur. Should the availability of chemical pesticides decline sharply for any reason, then the use of nematode products could expand to fill the void.

For control of certain insects, nematodes may replace chemical pesticides, and in other cases they will be used in conjunction with them. While it is highly improbable that entomopathogenic nematodes will ever capture a predominant share of the

pest control market, they definitely have a place in pest control and their market share will continue to increase.

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