INTRODUCTION OF ENTOMOPATHOGENIC NEMATODE PRODUCTS INTO LATIN AMERICA AND THE CARIBBEAN[†]

Ramon Georgis and Albert Hom

Biosys, Inc, 1057 East Meadow Circle, Palo Alto, CA 94303, U.S.A.

ABSTRACT

Georgis, R., and A. Hom. 1992. Introduction of entomopathogenic nematode products into Latin America and the Caribbean. Nematropica 22:81–98.

During the last decade, there has been heightened interest in augmentative biological control of insects by utilizing inundative releases of insect-parasitic nematodes in the families Steinernematidae and Heterorhabditidae. These nematodes and their associated entomopathogenic bacteria satisfy essential criteria for augmentative biological control. Rapidly increasing knowledge regarding biology, host range, and epidemiology has laid groundwork for the eventual use of these nematodes as effective biological control agents throughout the world. Concurrently, significant advances in large-scale production, formulation, and application methods have been achieved by commercial producers of nematode-based products, which are marketed for biological control of insects through the technique of inundative release in North America, Australia, China, and Europe. Commercial firms producing entomopathogenic nematodes may be able to make important contributions to the development of pest management strategies in Latin America and the Caribbean by providing nematodes for multiple field trials designed to investigate the biology and efficacy of both indigenous and exotic species. Training acquired by local researchers during these trials could accelerate the acquisition of essential information about biological factors affecting successful utilization of entomopathogenic nematodes as biological control agents. Ultimately, the training could be transferred to users of nematode-based products in commercial pest management programs.

Key words: biological control, commercialization, entomopathogenic nematodes, Heterorhabditidae, insects, marketing, Steinernematidae.

RESUMEN

Georgis, R. y A. Hom. 1992. La introducción de productos conteniendo nematodos entomopatogénicos en Latino América y el Caribe. Nematrópica 22:81–98.

Durante la última década, ha crecido el interés en el control biológico de insectos usando la liberación masiva de nematodos entomoparásitos de las familias Steinernematidae y Heterorhabditidae. Estos nematodos y las bacterias entomopatogénicas asociadas con ellos, satisfacen criterios esenciales para el control biológico aumentativo. Un incremento rápido del conocimiento de la biología, rango de hospederos y epidemiología, ha proveído un base para el uso eventual de estos nematodos como agentes eficaces del control biológico de insectos alrededor del mundo. A la vez, significativos avances en su producción en gran escala, formulación y métodos para su aplicación han sido logrados por los productores comerciales de productos conteniendo nematodos, los cuales son vendidos para el control biológico de insectos por medio de la técnica de la liberación masiva en Norte América, Australia, la China y Europa. Las compañías comerciales productores de nematodos entomopatogénicos quizás pudieran realizar contribuciones importantes al desarrollo de estrategias para el manejo de plagas en Latino América y el Caribe, proporcionando nematodos para múltiples ensayos de campo diseñados para evaluar la biología y eficacia tanto de las especias indígenas como de las foráneas. La instrucción práctica adquirida por investigadores locales durante estos ensayos podría acelerar la acumulación de información esencial concerniente a los factores que afectan la utilización exitosa de nematodos entomopatogénicos como agentes de control biológico. Por útilmo, la instrucción podría ser transferida a los usuarios de productos conteniendo nematodos en programas comerciales de manejo de plagas.

[†]This paper was presented as part of an invitational symposium, "Biological Control of Insect Pests with Entomopathogenic Nematodes," at the XXIII Annual Reunion of ONTA, San Antonio, Texas, U.S.A., 21–27 July 1991.

Palabras clave: comercialización, control biológico, Heterorhabditidae, insectos, mercadotecnia, nematodos entomopatogénicos, Steinernematidae.

INTRODUCTION

Extensive use of chemical pesticides has resulted in widespread insect resistance to pesticides and adverse effects on beneficial insects, wildlife, and human health throughout the world. In response, there has been increased demand for alternative and selective pest control agents, in particular, biological controls. In general, three kinds of biological control are recognized: natural, classical, and augmentative. Natural control is that population regulation which occurs in the habitats that co-evolving plants, predators, and prey occupy. Classical control involves the introduction, by man, of natural enemies to control both native and exotic pests. Augmentative control involves the manipulation, by man, of populations of natural enemies by altering environmental conditions, by aiding dispersal through inoculative releases of small numbers of individuals, or by greatly augmenting natural enemy populations to obtain immediate pest suppression through inundative releases of massive numbers of individuals (14,21,49).

During the last decade, there has been heightened interest by university, governmental, and industrial scientists in augmentative biological control of insects using entomopathogenic nematodes belonging to the families Steinernematidae and Heterorhabditidae (Table I). The nematodes are mutualistically associated with insect-pathogenic bacteria in the genus *Xenorhabdus*. These nematode-bacterium associations meet many criteria for augmentative control of insects through inundative releases including: broad host range; ability to kill hosts rapidly; a durable infective stage capable of storage, distribution, and persistence; available inexpensive mass-production technologies; no evidence of insect immunity; safety to plants and vertebrates; and application with existing spray equipment (45,68). Advances in large-scale production, formulation, and application methods have persuaded several companies in Europe, Australia, and North America to explore the economic aspects of development and sale of these nematode-based products (29).

Nematodes have been successfully applied to control agricultural and horticultural insect pests in many countries. In China, various species of *Steinernema* and *Heterorhabditis* have been used against orchard and ornamental tree pests such as the peach fruit borer (*Carposina nipponensis*) or yellow spotted willow borer (*Anaplophora glabripennis*) (65,89). In Europe, nematode-based products are available primarily for the control of

Table 1. Recognized Species of *Steinernema* and *Heterorhabditis*.[z]

Species	Original source
Steinernema affinis Bovien	Bibionidae (*Bibio* sp.)
S. anomali Kozodoi	*Phyllophaga* sp.
S. carpocapsae Weiser	*Cydia pomonella*
S. feltiae Filipjev	*Agrotis feltiae*
S. glaseri Steiner	*Popillia japonica*
S. intermedia Poinar	Soil
S. kushidai Mamiya	*Anomala cuprea*
S. rara Doucet	Soil
S. scapterisci Nguyen & Smart	*Scapteriscus vicinus*
S. ritteri Doucet & Doucet	Soil
Heterorhabditis bacteriophora Poinar	*Heliothis punctigera*
H. megidis Poinar, Jackson, & Klein	*Popillia japonica*
H. zealandica Wouts	Scarabaeidae

[z]Modified from Poinar (71).

black vine weevil (*Otiorhynchus sulcatus*) and sciarid flies (*Sciaria* spp.) in mushrooms and horticultural crops (10,15,29). In the United States and Canada, commercial products are sold to control a wide spectrum of soil and cryptic-inhabiting insects including the black vine weevil and cranberry girdler (*Chrysoteuchia topiaria*) in cranberry bogs, citrus weevils (*Diaprepes abbreviatus* and *Pachnaeus litus*) in citrus groves, black cutworm (*Agrotis ipsilon*), sod webworm (*Parapediasia* spp.), and billbugs (*Sphenophorus* spp.) in turfgrass, and black vine weevil and sciarid larvae in greenhouses (26; and biosys, unpublished data).

The entomopathogenic nematodes have not been fully exploited in Latin America and the Caribbean partly because they have not been available in quantities needed for adequate testing outside of the laboratory. However, numerous local and economically important insect pests of cotton, corn, soybean, citrus, coffee, and sugarcane are susceptible to the nematodes. The need for alternatives to chemical pesticides is increasing due to demand for decreased pesticide residues in high value crops for export. The potential value of biopesticides in integrated pest management programs is also great in Latin America and the Caribbean, but so far only *Bacillus thuringiensis* has been exploited to any extent (77).

HISTORICAL PERSPECTIVE

Prominent nematologists from Latin America and the Caribbean have contributed significantly to the taxonomy and discovery of entomopathogenic nematodes. One of the pioneer nematologists, I. Travassos from Brazil, placed *Aplectana kraussei* in a new genus and named it *Steinernema* (93). *Heterorhabditis hambletoni* was originally described from a parasitized cotton borer (*Eutinobothrus brasiliensis*) and was placed in the genus *Rhabditis* by C. Pereira in 1937 (67). Although it was not noted at the time, this was the first account of the new genus *Heterorhabditis*. Contemporary researchers are represented by M. Doucet and P. Stock of Argentina, G. R. Kloss and H. G. Fowler of Brazil, R. Alatorre-Rosa of Mexico, A. Kermarrec of Guadeloupe, A. Landszabal of Columbia, W. Figueroa and J. Roman of Puerto Rico, and J. M. Allard of Trinidad.

Many steinernematids and heterorhabditids have been recovered from insect pests and soil samples in Latin America and the Caribbean (Table 2). The strain, *Steinernema carpocapsae* Mexican, was originally recovered from infected codling moth larvae (*Cydia pomonella*) in Allende, Chihuahua, Mexico, by L. Caltagirone (67). An Argentine strain of *S. carpocapsae* was collected from an alfalfa pest, *Graphognathus leucoloma*, in Baliroche, Argentina (71). In Santa Rosa, Brazil, *S. glaseri* Araros strain was isolated from *Migodulus fryanus* (71).

Three newly described steinernematid species were recently discovered in Argentina and Uruguay. *Steinernema rara* (17) was collected from *Heliothis* larvae in Córdoba, Argentina. *Steinernema scapterisci* was recovered in Uruguay (61) and Argentina (P. Stock, personal communication) from mole crickets (*Scapteriscus* spp.). *Steinernema scapterisci* appears exceptionally well adapted for parasitizing mole crickets (37,62). The newly discovered species, *Steinernema ritteri* (18), was isolated from soil samples from Río Cuarto, Córdoba, Argentina, and its host range remains to be determined.

Heterorhabditid species associated with important insect pests have been found in Cuba, Brazil, and Argentina (35,71). *Heterorhabditis bacteriophora* (= *heliothidis*) Tetuan and P_2M strains were

Table 2. Steinernematid and heterorhabditid nematodes recovered from Latin America and the Caribbean.

Species	Strain	Source	Geographic location
Steinernema carpocapsae	Mexican	*Cydia pomonella*	Allende, Mexico
	Argentina	*Graphognathus leucoloma*	Argentina
S. glaseri	Araros	*Migdoulus fryanus*	Santa Rosa, Brazil
S. rara	Córdoba	*Heliothis* sp.	Río Cuatro, Argentina
S. scapterisci	Uruguay	*Scapteriscus vicinus*	Uruguay
	Argentina	Soil	Argentina
S. ritteri	N/A[z]	Soil	Río Cuatro, Argentina
Heterorhabditis bacteriophora	Agrl	Soil	Río Cuatro, Argentina
	María	Soil	Río Cuatro, Argentina
	Brazil	Soil	Pernambrico, Brazil
Heterorhabditis spp.	Tetuan	*Cylas formicarius*	Artenisa, Cuba
	P_2M	*Pachneus litus*	Havana, Cuba

[z]Not applicable; no strain designation available.

collected from parasitized larvae and pupae of sweet potato weevil (*Cylas formicarius*) and blue green weevil (*Pachnaeus litus*) (35). The Brazil strain of *Heterorhabditis* sp. was recovered from the hot arid soil in Pernambrico, Brazil, (71) and the Argl strain was isolated from soil samples in Río Cuarto, Córdoba, Argentina (71).

COMMERCIAL DEVELOPMENT

One of the most important factors in entomopathogenic nematode product development is the feasibility of producing the nematodes at an acceptable cost and in sufficient quantities, first for use in field testing, and later for product commercialization. During the past decade, significant progress in large scale production, especially in an *in vitro* process for steinernematid species, has enabled commercial companies to produce nematodes more efficiently and inexpensively (27). In the United States and Canada, steinernematid-based products are cost effective and comparable to standard chemical insecticides in high and medium cash crops such as cranberry, citrus, ornamentals, and turfgrass. Heterorhabditids are reliably produced in solid media culture, but large-scale, consistent production in liquid fermentation of this nematode lags behind steinernematids (36); further technological advancements are needed to decrease production costs.

Improvements in formulation have resulted in nematode products that have a longer shelf life and stability and are easier to mix and apply (27). Infective juveniles of steinernematids and heterorhabditids can be immobilized or partially desiccated on carriers, such as clay, polyacrylamide, and alginate gels, that promote nematode survival during transit and storage (29). Currently, commercially available steinernematid-based alginate gel products have a shelf life of up to 5 months at room temperatures. In contrast, heterorhabditids require continu-

ous refrigeration to maintain product stability (Table 3).

MARKET OPPORTUNITIES AND PESTS OF ECONOMIC IMPORTANCE

The commercial potential of entomopathogenic nematodes in Latin America and the Caribbean appears to be very high. Numerous insect pests are suitable targets (Table 4). Moreover, the rainy, humid climate in much of Latin America and the Caribbean provides a favorable environment for nematode application to soil and foliar insects (7). The most likely users of nematode-based products are large plantation growers, for example, of sugarcane and coffee, and relatively prosperous farmers growing cash crops (fruits, vegetables, and flowers) for local and export markets. A strong incentive for growers in Latin America and the Caribbean to reduce chemical pesticide usage is the increasing demand in countries that import agricultural products from the region for less pesticide residue in food.

Encouraging trials with nematodes have been against insects that spend a portion of their life cycles in the soil or in moist, protected habitats. In soil, nematodes have an advantage over chemical pesticides by virtue of their host-seeking ability (1). In protective cryptic habitats, the nematodes are sheltered from environmental extremes, such as high temperatures and ultra-violet radiation, that have deleterious effects on survival and infectivity (23).

Wassink and Poinar (90) listed 97 species of Latin American insects from 11 orders as susceptible hosts to *S. carpocapsae*. This broad host range was based primarily on infections achieved under laboratory conditions that precluded behavioral or ecological barriers to infection (44). The following discussion will highlight the results of both laboratory and field trials with nematodes against important insect pests. Some of these tests were conducted outside of Latin America and the Caribbean, but involved insects found in these regions.

Table 3. Current commercial formulations of steinernematid and heterorhabditid nematode-based products.

Formulation	Storage period		Product description
	Shelf-life[y]	Refrigeration[z]	
Alginate gel	5 months	6 months	4.0-liter container, 250×10^6 steinernematid IJ entrapped in a gel matrix and coated on a mesh screen
Clay	—	3 months	50×10^6 heterorhabditid IJ nematodes on clay enclosed in a bag (15 × 20 cm)
Polyacrylamide	5 days	6 months	3×10^9 steinernematid IJ per box; each 1×10^9 entrapped into 2.3 kg net gel weight of gel matrix enclosed in a mesh screen bag
Flowable gel	3 months	6 months	250×10^6 steinernematid IJ suspended in a gel material enclosed in a bag (40 × 50 cm)

[y]20–28 C; not recommended for clay formulation.
[z]2–10 C.

Table 4. Proposed markets for steinernematid and heterorhabditid-based products in Latin America and the Caribbean.

Crop	Common name	Scientific name	Target stage[y]	Application[z]
Banana	Banana root borer	*Cosmopolites sordidus*	N	S
Citrus	Blue green weevil	*Pachneus litus*	I	S
	Sugarcane rootstalk borer	*Diaprepes abbreviatus*	I	S
	Sap beetle	*Carpophilus fumatus*	I	S
	Mediterranean fruit fly	*Ceratitis capitata*	I	S
	Fruit flies	*Anastrepha* spp.	I	S
		Toxotrypana curvicauda	I	S
Coffee	Coffee berry borer	*Hypothenemus hampei*	I	A,S
	White grubs	*Phyllophaga menetriesi*	I	S
Corn	Weevil	*Listronotus dietrichi*	I	S
	White grubs	*Phyllophaga* spp.	I	S
	Black cutworm	*Agrotis ipsilon*	I	S
	Fall armyworm	*Spodoptera frugiperda*	I	A
	Corn rootworm	*Diabrotica* spp.	I	S
Cucumber	Melonworm	*Diaphania hyalinata*	I	A
	Pickleworm	*D. nitidalis*	I	A
	Cutworms	*Spodoptera* spp.	I	A
Greenhouse and nursery plants	Beet armyworm	*Spodoptera exigua*	I	A
	Black vine weevil	*Otiorhynchus sulcatus*	I	S
	Leafminer	*Liriomyza trifolii*	I	A
	Sciarid flies	*Sciarid* sp.	I	A
	Strawberry root weevil	*Otiorhynchus ovatus*	I	S
Beans and other legumes	Cutworms	*Agrotis* spp.	I	S
	Armyworms	*Spodoptera* spp.	I	A
	Tobacco budworm	*Heliothis virescens*	I	S
	Lesser cornstalk borer	*Elasmopalpus lignosellus*	I	S
	Cucumber beetle	*Diabrotica* spp.	I	S
	White grubs	*Phyllophaga* spp.	I	S
Mushroom	Phorid fly	*Megaselia halterata*	I	S
	Sciarid fly	*Lycoriella mali*	I	S
Peanut	Granulate cutworm	*Feltia subterranea*	I	A
	Lesser cornstalk borer	*Elasmopalpus lignosellus*	I	S
	Corn rootworm	*Diabrotica* spp.	I	S
Potato	Fall armyworm	*Spodoptera frugiperda*	I	A
	Colorado potato beetle	*Lepinotarsa undecimlineata*	I	S
	White grubs	*Phyllophaga* spp.	I	S
Rice	White grubs	*Phyllophaga* spp.	I	S
	Fall armyworm	*Spodoptera frugiperda*	I	A
	Mole crickets	*Scapteriscus* spp.	I	S

Table 4. (continued).

Crop	Common name	Scientific name	Target stage[y]	Application[z]
Sorghum	Fall armyworm	*Spodoptera frugiperda*	I	A
	Corn rootworm	*Diabrotica* spp.	I	S
Sugarcane	Mexican rice borer	*Eoreuma loftini*	I	A
	Sugarcane froghopper	*Aenelomia* spp.	I	A
	Sugarcane borer	*Diatrea saccharalis*	I	A
	White grubs	*Ligyrus subtropicus*	I	S
	Lesser cornstalk borer	*Elasmopalpus lignosellus*	I	S
Sweet potato	Sweet potato weevil	*Cylas formicarius*	I	S
	White grubs	*Phyllophaga* spp.	I	S
Tomato	Tomato hornworm	*Manduca sexta*	I	A
	Armyworm	*Spodoptera* spp.	I	A
	Tomato pinworm	*Keiferia lycopersicella*	I	A
Turfgrass	Mole crickets	*Scapteriscus* spp.	N	S
Urban	Housefly	*Musca domestica*	N	A
	German cockroach	*Blatella germanica*	N	A
	Wasp	*Vespula* spp.	N	A
Vegetable and field crops	Cutworms	*Agrotis* spp.	I	A
	Cucumber beetles	*Diabrotica* spp.	I	S
	Flea beetles	Chrysomelidae	I	S

[y]N = nymphs and (or) adults using bait stations; I = immatures.
[z]S = soil applications; A = above-ground applications in cryptic or greenhouse environments.

Medically Important Insect and Invertebrate Pests

Many medically important insect and invertebrate pests are susceptible to both steinernematid and heterorhabditid nematodes: conenose bugs (*Rhodnius prolixus* and *Triatoma infestans*) (66); a tick, *Boophilus annulatus* (81); the rat flea (*Xenopsylla cheopis*) (58); mosquitoes (*Aedes* spp. and *Culex* spp.) (56,72); black flies (*Simulium* spp.) (24); fire ants (*Solenopsis* spp.) (41); cockroaches (*Blattella* spp.) (55; and biosys, unpublished data); and the house fly (*Musca* spp.) (25,59). Chinese scientists have found that the snail, *Oncomelania jupensis*, which is the intermediate host of the blood fluke, *Schistosoma japonicum*, is also susceptible (51). However, various ecological factors, such as spatial separation of host and nematode, host defense mechanisms, and hostile habitats, are obstacles to the implementation of entomopathogenic nematodes against these hosts. Alternatively, incorporating nematodes in bait stations holds promise against adult cockroaches (29) and the adult house fly (78).

Agricultural Insect Pests

Mole crickets, Scapteriscus *spp. (Orthoptera: Gryllotalpidae):* Several species of mole crickets that were originally introduced from South America have become major pests of turf and pasture grasses in the southern United States. In 1985, scientists from the University of Florida

searched for natural enemies of mole crickets in South America. They found several steinernematids that are well adapted to mole cricket parasitism. In laboratory bioassays comparing virulence of Steinernema isolates against mole crickets, an Uruguayan strain of an unidenitified *Steinernema* species was selected as the most promising for release in Florida. Subsequent morphological and biological studies of this nematode revealed that it was a new species and it was appropriately named *Steinernema scapterisci* (61).

In field release trials in Florida, *S. scapterisci* provided 74.8% reduction in mole cricket damage at the application rate of 2.5×10^9 infective juveniles (IJ) per hectare. The standard chemical, Acephate, at 3.40 kg a.i./hectare caused a 68.4% reduction (29). Late stage nymphs and adult mole crickets are found to be highly susceptible to the nematodes, whereas younger nymphs are more resistant to nematode infection in the field (37).

Sugarcane froghopper, Aenelomia *spp. (Hemiptera: Cercopidae):* This insect is one of the most serious pests of sugarcane in Mexico, Central America, Venezuela, Trinidad, Brazil, and Argentina. Nymphal and adult feeding on the plant leads to stunted growth, loss of yield, and a reduction in juice quality (3). Steinernematid and heterorhabditid nematodes have potential as part of an IPM strategy against the sugarcane froghopper. In 1986, in small-scale cage experiments, *S. carpocapsae* Mexican strain was found to be highly pathogenic to the nymphs and adults. The nematode survived in sand for up to 4 weeks and moved through 10 cm of compacted clay soil to locate a host (4). A native heterorhabditid species has also been isolated in Trinidad and its potential to infect sugarcane froghopper is currently being evaluated (4).

Banana root borer weevil, Cosmopolites sordidus *(Coleoptera: Curculionidae):* Plantains and bananas are the most important starchy crops in Puerto Rico and the Virgin Islands. The larvae bore tunnels into the corms of banana plants rendering the plant susceptible to secondary pathogen attack, which leads to fruit decay and poor yield (20). Most chemicals are ineffective because they cannot reach the larva where it feeds. In greenhouse studies, *S. carpocapsae, S. feltiae,* and *S. glaseri* at dosage rates of 400, 4 000, and 40 000 IJ per plant reduced the number of tunnels produced by the sixth and seventh instar larvae. Mortality reached 50%, which was considered satisfactory due to the difficulties in reaching the larvae (20). Treverrow and Bedding (88), using *S. carpocapsae* All strain applied to freshly made cavities in residual rhizomes, reduced the larval population by up to 70% compared to untreated plants. The placement and type of cavity affected treatment efficacy.

Sweet potato weevil, Cylas formicarius *(Coleoptera: Curculionidae):* The sweet potato weevil is the most serious insect pest attacking sweet potato. Both the adults and larvae cause damage on fleshy roots and vines. Weevil feeding can result in vine malformation, reduce plant vigor, and cause a paling of the leaves. An entire crop can be lost due to weevil damage. Preliminary studies have indicated that *H. bacteriophora* is more efficacious than *S. carpocapsae* in reducing weevil damage (39). In a Florida experiment, *H. bacteriophora* Hp88 did not consistently reduce pest density, but did reduce weevil damage to sweet potato storage roots compared with untreated plants (40). Weevil damage was intermediate on

plants treated with a combination of two chemical insecticides, methamidophos and endosulfan. Additional studies showed that *H. bacteriophora* persisted in soil for over 250 days in the field.

Sugarcane rootstalk borer, Diaprepes abbreviatus, *and blue-green weevil,* Pachnaeus litus *(Coleoptera: Curculionidae):* These insects are the major curculionid pests of citrus in the West Indies, South America, and Florida. The adult weevil attacks orange and grapefruit foliage; the soil-inhabiting larva feeds on the roots. Current control is limited to the application of insecticides to reduce the adult population. Indigenous steinernematid and heterorhabditid nematodes are infectious to *D. abbreviatus* in Florida citrus groves (6). In subsequent laboratory and field trials, *S. carpocapsae, S. glaseri,* and *H. bacteriophora* provided significant control of *D. abbreviatus*, but have short residual action. In an effort in increase nematode persistence, *S. carpocapsae* was added to a starch polymer water-retention agent and mixed with water, then applied to the soil surface. The addition of the water-retention agent enhanced nematode infection of insect larvae for more than 2 months (82). Currently, a commercially available nematode product is marketed as an economical and environmentally acceptable method to manage root weevil larvae in Florida. Nematodes are added to irrigation systems, resulting in a 72–98% reduction of the emergence of *D. abbreviatus* and *P. litus* adults (29).

White grubs (Coleoptera: Scarabaeidae): The larval stage of the Scarabaeidae, collectively referred to as white grubs, are important pests of turfgrass, pasture, sugarcane, citrus, and forests throughout the world (48). Millions of dollars are spent annually to control these insects in turfgrass. Most of the preliminary nematode trials have been conducted against white grubs in sugarcane plantations. Under laboratory conditions, *S. glaseri* was most effective (100%) against the third instar of *Ligyrus subtropicus* (85). In small field tests, half of the white grubs collected from treatment areas were infected with *S. glaseri* (86). However, more recent field releases of *S. glaseri* failed to significantly reduce the number of larvae, although *S. glaseri* appeared to be more effective than *S. carpocapsae* (87). *Heterorhabditis bacteriophora* has been shown to be more efficacious than *S. carpocapsae* in controlling Japanese beetle grubs (*Popillia japonica*) in turfgrass (28,48,73). Season of application, soil temperature (> 20 C), soil type, irrigation frequency, and thatch depth influence the nematode's ability to locate and infect the larvae.

Coffee berry borer, Hypothenemus hampei *(Coleoptera: Scolytidae):* The coffee berry borer is an introduced pest in Latin America and the Caribbean. Coffee crop losses are caused by feeding of the female in two ways: 1) it bores into the berries and causes severe damage to the nearly ripe or ripe fruit, thereby destroying the developing bean; and 2) green berries are partially bored, resulting in the premature drop and decay of the berry (60). In a laboratory experiment, a native *Heterorhabditis* species entered infested berries and caused significant mortality of adult and larval berry borers (5). Unfortunately, difficulties with nematode desiccation and persistence under field conditions prevented further investigation. Since other researchers have achieved partial success using evaporative retardants to prolong nematode persistence on foliar surfaces, perhaps the potential for using nematodes for suppression of the coffee borer should be reconsidered. Alterna-

tively, the larvae overwinter in fallen berries on the ground and it may be feasible to use entomopathogenic nematodes in an IPM program as a preventative spray to eliminate a portion of the emerging adults of the next generation.

Fruit flies (Diptera: Tephritidae): Several species of fruit flies in the genera *Anastrepha, Toxotrypana,* and *Ceratitis* are serious pests of fruits and vegetables in Latin America (20). Damage is caused by the adult ovipositing into the fruit or vegetable and the larvae boring into the flesh of the developing fruit. When feeding ceases, the mature third-instar larva exits the fruit to pupate in the shallow soil surrounding the base of the host plant. It is during this soil-dwelling developmental stage that the larva can come into contact with the nematodes and be infected. Initial laboratory and field experiments indicate that the larvae are highly susceptible, the pupae in puparia are not susceptible, and the adult flies are marginally susceptible (52).

In Hawaiian field trials, an average of 87.1% larval mortality of the Mediterranean fruit fly, *C. capitata,* was observed following applications of *S. carpocapsae* Mexican strain at 500 IJ/cm^2 soil (53). Currently, researchers are examining lower nematode dosage rates for economic control of the Mediterranean fruit fly.

Mexican rice borer, Eoreuma loftini *(Lepidoptera: Pyralidae):* Despite its common name, this insect is a serious pest of sugarcane in Texas and Mexico. The larvae crawl between the leaf sheath and the stalk, mine the leaf sheath for 2–3 weeks, and then bore into the sugarcane stalk (79). The interstitial space within the sheath fold collects water and so provides a wet and protected environment for the nematodes. In a laboratory bioassay, third and fourth instar larvae exposed to *S. carpocapsae, S. feltiae,* and *H. bacteriophora* provided larval mortality of 82.5, 74.0, and 44.5%, respectively; hence, *S. carpocapsae* and *S. feltiae* are considered to be potential biological control agents of this pest (79).

APPLICATION CONSIDERATIONS AND LIMITATIONS

In some situations, a target insect's behavioral, physical, or physiological defense mechanisms may affect the nematode's effectiveness (1). For example, when nematodes are applied to termite colonies, the workers are able to recognize infected individuals and isolate them behind earthen barriers (73). Similarly, fire ants (*Solenopsis* spp.) display avoidance behavior and move their colonies elsewhere as a result of nematode treatment (41). Morphological defenses include sieve plates in scarabaeid species, which prevent nematode access to the hemocoel (1). Physiological defenses in chrysomelid beetles and mosquitoes frequently result in encapsulation and melanization of infective juveniles after penetrating the hemocoel (56,69).

Despite effectiveness against insects in soil and protected habitats, attempts to use nematodes to control insects in foliar and aquatic habitats have been discouraging. In most cases, foliar applications of nematodes have caused a statistically significant decrease in pest density. However, pest reduction has not been sufficient to reduce crop damage below economic thresholds (91). When applied to exposed surfaces, the nematodes require high ambient humidity (> 90%) and free water on the leaf for survival (9). Efforts to extend nematode survival with the additions of evaporetardants and UV protectants have achieved partial success against leafminers (*Liriomyza* spp.) and the corn

earworm (*Heliothis* spp.) (32; and A. Hara, personal communication).

Filth-breeding insects, *e.g.*, flies and beetles in manure, would appear to be good candidates for control with entomopathogenic nematodes (9). However, many attempts to control these insects have failed due to hostile environments. High temperatures (38 C), predators, and toxic ammonia and salts, account for poor nematode persistence in manure (30,92). In aquatic habitats, a number of factors, including damage to the nematode during ingestion, host-evoked immune response, rapid settling rates, low oxygen levels, and spatial separation of host and nematode, have limited the use of nematodes against medically important insects, such as mosquitoes and black flies, as well as agricultural pests like the rice water weevil (*Lissorhoptrus oryzophilus* (15,24,56,72).

Although many possibilities exist for the use of entomopathogenic nematodes in Latin America and the Caribbean, successful pest suppression will not be achieved without a thorough understanding of various components including: identifying a pest problem amenable to nematode control; determining the most efficacious nematode strains; assessing the environmental constraints; and developing application techniques necessary to obtain optimum distribution for control.

Entomopathogenic nematodes are not a panacea for all insect pests. As was mentioned before, host defense mechanisms and behavior, and the duration of contact between the host and the nematode are important factors affecting nematode efficacy against certain insects. For example, timing is critical when applying nematodes against corn rootworms (*Diabrotica* spp.) (27; and C. Redmond, personal communication). The second larval instar is the target stage but is present only for 1–2 weeks. So far, no degree-day model or sampling methods have been developed that can accurately predict when the target stage will be present in the soil. It is difficult, therefore, to time the nematode application to coincide with the presence of the target larval stage.

Steinernematid and heterorhabditid species are not equally effective against all target insects (12); each species has a number of preferred insect hosts. In general, steinernematids are most effective against insect pests found near the soil surface and, therefore, may be best adapted to attacking insects such as the mole crickets and cutworms, which feed at the soil-litter interface or on the soil surface (27,57). In most cases, *S. glaseri* has demonstrated superiority over other steinernematids and heterorhabditids against scarabaeid larvae (48,74,85,86). Recent data indicate high performance of *S. feltiae* (= *bibionis*) against dipterans, especially sciarids and mushroom flies (29; and A. Hom, unpublished data). *Heterorhabditis* spp. are more effective against insects that occur relatively deep in soil, such as the Japanese beetle (74) and sweet potato weevil (40), due to superior host-seeking abilities and a tendency to disperse downward.

The relatively poor migration of nematodes needs to be taken into account when developing application methods (1,12,27). When applied to the soil surface, the majority of the nematodes remain at the point of placement with little lateral or vertical dispersal (48,57). This limited dispersal puts the nematodes at risk of adverse exposure to UV radiation and rapid desiccation. Soil surface spraying is still the preferred method because it is quick and easy, and provides good coverage. Therefore, high nematode concentrations are needed to ensure that

a sufficient number of nematodes are distributed near the target insect. Other methods of delivering nematodes to the soil include injection, dipping root stock, and application through irrigation systems (27).

Environmental conditions are known to have important effects on the efficacy of currently available commercial formulations of entomopathogenic nematodes. The following guidelines, which have been developed for these products should be taken into account when doing field applications.

1. Soil temperatures ranging from 18–30 C are optimal for nematode survival, movement, and host infection. If the soil temperature is below 18 C, the nematode application should be postponed until soil is warmer (27,46).
2. Nematodes should be applied to moist soil. A general rule is not to apply nematodes under conditions that produce wilting in plants (68). Pre- and post-application irrigation and continuing moderate soil moisture after application are necessary to ensure nematode survival and persistence.
3. Nematode applications should be made during early morning or later in the evening to avoid the effects of ultraviolet radiation and temperature extremes.
4. A high spray volume is needed for nematodes to reach the depth occupied by the target insect. Depending on soil and turf conditions, 1400–1870 L/ha followed immediately with irrigation is acceptable (27).
5. Field application concentrations of 2.5–7.5 billion IJ/ha are in general required to provide adequate and consistent pest control (29). A high concentration is needed to overcome the negative impacts of predators, pathogens, and other ecological factors (38,46,67,74).
6. Repeat applications may be needed for acceptable control when the soil environment is hostile to the nematodes, or when target insects have multiple or overlapping generations (*e.g.*, sciarid flies, black cutworm, mole crickets).
7. For certain insects, such as white grubs and root weevils, field trial durations of 4 weeks or longer may be necessary for a significant reduction in the insect population to occur (83). For lepidopterans, 3–7 days is generally sufficient for maximum control.

SAFETY AND EFFECTS ON NON-TARGET ORGANISMS

The steinernematids and heterorhabditids, and their symbiotic bacteria, *Xenorhabdus* spp. are exempt from the registration requirements of the Federal Insecticide, Fungicide, Rodenticide Act (FIFRA) in the United States. Thus far, no standard laws and regulations have been enacted in Latin America and the Caribbean countries governing the field release of entomopathogenic nematodes.

Laboratory tests on rats (50), mice and chicks (75), and guinea pigs (64) have demonstrated no adverse effects to these vertebrates when exposed to entomopathogenic nematodes. Among tested vertebrates, only young tadpoles were adversely affected when exposed to the nematodes (70,71). In this case, foreign bacteria (not *Xenorhabdus* spp.) that entered the tadpole through the penetration hole made by the nematodes were the primary cause of death. Earthworms were not significantly affected by the nematodes. Nematodes were able to reproduce on earthworm cadavers but did not kill intact worms and did not significantly increase mortality in damaged worms (2,11,63).

Kaya (43) and Akhurst (2) summarized available information on the effects of entomopathogenic nematodes on beneficial insects. They concluded that nematodes currently used for biocontrol of pest insects pose few problems for beneficial insects. Additional laboratory and field studies confirmed the absence of detrimental effects on non-target insects when entomopathogenic nematodes were used for short term control of insect pests (31,38).

FUTURE PROSPECTS

We think that established commercial producers of entomopathogenic nematode

products in Europe, Australia, and North America could make an important contribution to pest management in Latin American and the Caribbean by providing nematodes for inundative releases in multiple small-scale field trials. To be an attractive venture for the commercial nematode producer, the trials would need to be targeted initially toward one or a few, serious local insect pests. Such trials would generate essential efficacy data and also provide researchers at widely scattered locations with essential training and experience in various aspects of nematode application and efficacy evaluation. Coordination of multiple trials by a single firm would permit the adoption of a standard experimental protocol to facilitate comparisons between results obtained at various locations (7,28). Concurrently, considerable efforts could and should be made to search for indigenous steinernematid and heterorhabditid nematodes. The *Galleria* trap method (8) has proven well suited to such collections at numerous locations. In some cases, indigenous nematodes may be far better suited than exotic species for inundative releases against native insects because of selective adaptation to those insect hosts, to local climate, and to biological population regulators (7). The trials would best be conducted in collaboration with agricultural university researchers, who would be in a position to transfer the training obtained during the trials to eventual users of products marketed by commercial entomopathogenic nematode producers, should such markets develop in the future.

Considerable additional information will be needed regarding the biology and potential efficacy of indigenous and exotic entomopathogenic nematodes in Latin America and the Caribbean, and regarding the applicability of existing commercial formulations, before widespread utilization of these organisms to control insects pests can be successful. Given that the needed biological information were in place, there would be three possible sources of entomopathogenic nematode products for use in augmentative biological control applications: 1) local cottage industries that could mass produce nematodes using *in vivo* culture; 2) local facilities built to produce and formulate nematodes on a large scale on solid and liquid culture media; 3) established entomopathogenic nematode producers in Europe, Australia, or North America, which already produce nematodes on solid and liquid culture for distribution and sale throughout the world. Mass production by local cottage industries using *in vivo* methods (16,54) would require only inexpensive local raw materials and labor, and all nematodes could be used immediately, thereby eliminating prolonged shelf life requirements. It should be noted, however, that *in vivo* methods are highly labor intensive, lack the economy of scale of solid and liquid culture methods (22), and are prone to contamination by potentially dangerous microorganisms (44). On the other hand, construction and maintenance of the comparatively sophisticated facilities required for large-scale solid and liquid culture locally may not be feasible in many cases due to requirements for large capital investment and highly specialized training. This suggests to us that significant markets for foreign, commercially produced nematode-based products may develop. Those markets could function through joint venture agreements with locally established agrichemical companies or corporate plantations in the region.

For local cottage industries, and for local and foreign large-scale producers of entomopathogenic nematode products, the development of markets will be a challenging task. Entomopathogenic

nematodes are generally more expensive to produce than are chemical larvicides in the quantities required for efficacy and require more training to use. Growers will be willing to pay the extra price and to learn to use a nematode-based product instead of an alternative chemical pesticide only if the nematode product has comparable efficacy when used at recommended rates, is stable and relatively easy to use, can be applied to a medium to high cash value crop, and the safety of the chemical pesticide is of concern. Large-scale producers can help solve the training problem by introducing products initially through researchers at agricultural universities, who in turn can train farm managers in proper methods for product use. Large-scale producers, in addition, are confronted with a product shelf life for currently available formulations of 5 months, which can be extended only by refrigeration. The shelf life problem probably can be addressed by careful scheduling of manufacture and distribution, as has been implemented successfully in the United States, Canada and western Europe.

A final opportunity worthy of further development is to incorporate entomopathogenic nematodes into integrated pest management programs. Entomopathogenic nematodes are generally compatible with most commercial chemical pesticides (33,34,80,94), as well as entomogenous fungi (42), bacteria (76), and viruses (47). The combination of entomopathogenic nematodes with chemical, biological, and cultural measures should result in an additive or supplemental mortality of the pest and a reduction in the amount of chemical needed for insect control (43).

ACKNOWLEDGEMENT

The authors thank Terry Weber, Denise Paterson, and Stephen Manweiler for their helpful suggestions and Dr. Grover Smart and Dr. Harry Kaya for their critical review of the manuscript.

LITERATURE CITED

1. AKHURST, R. J. 1986. Controlling insects in soil with entomopathogenic nematodes. Pp. 265–267 *in* R. A. Samson, J. M. Vlak, and D. Peters, eds. Fundamental and Applied Aspects of Invertebrate Pathology. Fourth International Colloquium of Invertebrate Pathology, Wageningen, The Netherlands.
2. AKHURST, R. 1990. Safety to nontarget invertebrates of nematodes of economically important pests. Pp. 234–238 *in* M. Laird, L. A. Lacey, and E. W. Davidson, eds. Safety of Microbial Insecticides. CRC Press: Boca Raton, Florida, U.S.A.
3. ALLARD, G. B. 1987. Prospects for the biocontrol of the sugarcane froghopper with particular reference to Trinidad. Biocontrol News and Information 8:105–115.
4. ALLARD, G. B., and C. A. CHASE. 1987. Unpublished report.
5. ALLARD, G. B., and D. MOORE. 1989. *Heterorhabditis* sp. Nematodes as control agent for coffee berry borer, *Hypothenemus hampei* (Scolytidae). Journal of Invertebrate Pathology 54:45–48.
6. BEAVER, J. B., C. M. McCOY, and D. T. KAPLAN. 1983. Natural enemies of subterranean *Diaprepes abbreviatus* (Coleoptera: Curculionidae) larvae in Florida. Environmental Entomology 12:840–843.
7. BEDDING, R. 1990. Logistics and strategies for introducing entomopathogenic nematode technology into developing countries. Pp. 233–246 *in* R. Gaugler and H. K. Kaya, eds. Entomopathogenic Nematodes in Biological Control. CRC Press: Boca Raton, Florida, U.S.A.
8. BEDDING, R., and R. AKHURST. 1975. A simple technique for the detection of insect parasitic rhabditid nematodes in soil. Nematologica 21:109–116.
9. BEGLEY, J. W. 1990. Efficacy against insects in habitats other than soil. Pp. 215–231 *in* R. Gaugler and H. K. Kaya, eds. Entomopathogenic Nematodes in Biological Control. CRC Press: Boca Raton, Florida, U.S.A.
10. BUGIANI, R. 1990. Nematodes against *Otiorhynchus* spp. Terra e Sole 45:575–576.

11. CAPINERA, J. L., S. L. BLUE, and G. S. WHEELER. 1982. Survival of earthworms exposed to *Neoaplectana carpocapsae* nematodes. Journal of Invertebrate Pathology 39:419–421.
12. CURRAN, J. 1990. Efficacy of entomopathogenic nematodes in field soils. Pp. 224–227 *in* Proceedings and Abstracts of the Vth International Colloquium on Invertebrate Pathology and Microbial Control, Adelaide, Australia, August 20–24, 1990.
13. DADD, R. H. 1971. Size limitations on the infectibility of mosquito larvae by nematodes during filter-feeding. Journal of Invertebrate Pathology 18:246–255.
14. DEBACH, P. 1974. Biological control by natural enemies. Cambridge University Press: New York, U.S.A. 323 pp.
15. DESEO, K. V. 1990. Entomopathogenic nematodes in plant protection. Informatore Fitopathologico 32:25–30.
16. DESEO, K. V., L. RUGGERI, and G. LAZZARI. 1990 Mass-production and quality control of entomopathogenic nematodes in *Galleria mellonella* L. larvae. P. 250 *in* Proceedings and Abstracts of the Vth Colloquium on Invertebrate Pathology and Microbial Control, Adelaide, Australia, August 20–24, 1990.
17. DOUCET, M. M. A. de. 1986. A new species of *Neoaplectana* Steiner, 1929 (Nematoda: Steinernematidae) from Córdoba, Argentina. Revue de Nématologie 9:317–323.
18. DOUCET, M. M. A. de, and M. E. DOUCET. 1990. *Steinernema ritteri* n. sp. (Nematoda: Steinernematidae) with a key to the species of the genus. Nematologica 36:257–265.
19. ESKAFI, F. M., and T. R. CUNNINGHAM. 1987. Host plants of fruit flies (Diptera: Tephritidae) of economic importance in Guatemala. Florida Entomologist 70:116–124.
20. FIGUEROA, W. 1990. Biocontrol of the banana root borer weevil, *Cosmopolites sordidus* (Germar), with steinernematid nematodes. Journal of Agriculture, University of Puerto Rico 74:15–19.
21. FLINT, M. L., and R. van den BOSCH. 1981. Introduction to Integrated Pest Management. Plenum Press: New York. 240 pp.
22. FRIEDMAN, M. J. 1990. Commercial production and development. Pp. 153–172 *in* R. Gaugler and H. K. Kaya, eds. Entomopathogenic Nematodes in Biological Control. CRC Press: Boca Raton, Florida, U.S.A.
23. GAUGLER, R. 1981. Biological control potential of neoaplectanid nematodes. Journal of Nematology 13:241–249.
24. GAUGLER, R., and D. MOLLOY. 1981. Field evaluation of the entomogenous nematode, *Neoaplectana carpocapsae*, as a biological control agent of black flies (Diptera: Simuliidae) Mosquito News 41:459–464.
25. GEDEN, C. J., R. C. AXTELL, and W. M. BROOKS. 1986. Susceptibility of the house fly, *Musca domestica* (Diptera: Muscidae) to the entomogenous nematodes *Steinernema feltiae, S. glaseri* (Steinernematidae) and *Heterorhabditis heliothidis* (Heterorhabditidae). Journal of Medical Entomology 23:326–333.
26. GEORGIS, R. 1990. Commercialization of steinernematid and heterorhabditid entomopathogenic nematodes. Pp. 275–280 *in* Brighton Crop Protection Conference, United Kingdom, November 19–22, 1990.
27. GEORGIS, R. 1990. Formulation and application technology. Pp. 173–191 *in* R. Gaugler and H. K. Kaya, eds. Entomopathogenic Nematodes in Biological Control. CRC Press: Boca Raton, Florida, U.S.A.
28. GEORGIS, R., and R. GAUGLER. 1991. Predictability in biological control using entomopathogenic nematodes. Journal of Economic Entomology 84:713–720.
29. GEORGIS, R., and N. G. M. HAGUE. 1991. Nematodes as biological insecticides. Pesticide Outlook 2:29–32.
30. GEORGIS, R., B. A. MULLENS, and J. A. MEYER. 1987. Survival and movement of insect nematodes in poultry manure and their infectivity against *Musca domestica*. Journal of Nematology 19:292–296.
31. GEORGIS, R., H. K. KAYA, and R. GAUGLER. 1991. Effect of steinernematid and heterorhabditid nematodes (Rhabitida: Steinernematidae and Heterorhabditidae) on nontarget arthropods. Environmental Entomology 20:815–822.
32. GLAZER, I., and A. NAVON. 1990. Activity and persistence of entomoparasitic nematodes tested against *Heliothis armigera* (Lepidoptera: Noctuidae). Journal of Economic Entomology 83:1795–1800.
33. HARA, A. R., and H. K. KAYA. 1982. Effects of selected insecticides and nematicides on the *in vitro* development of the entomogenous nematode *Neoaplectana carpocapsae*. Journal of Nematology 14:486–491.
34. HARA, A. R., and H. K. KAYA. 1983. Toxicity of selected organophosphate and carbamate pesticides to infective juveniles of the entomogenous nematode *Neoaplectana carpocapsae* (Rhabditida: Steinernematidae). Environmental Entomology 12:496–501.

35. HERNANDEZ, E. M. A., and Z. MRACEK. 1984. *Heterorhabditis heliothidis*, a parasite of insect pests in Cuba. Folia Parasitologica 31:11–17.
36. HOMINICK, W. M., and A. P. REID. 1990. Perspectives on entomopathogenic nematodes. Pp. 327–345 *in* R. Gaugler and H. K. Kaya, eds. Entomopathogenic Nematodes in Biological Control. CRC Press: Boca Raton, Florida, U.S.A.
37. HUDSON, W. G., and K. B. NGUYEN. 1990. Effects of soil moisture, exposure time, nematode age, and nematode density on laboratory infection of *Scapteriscus vicinus* and *S. acletus* (Orthoptera: Gryllotalpidae) by *Neoaplectana* sp. (Rhabditida: Steinernematidae). Environmental Entomology 18:719–722.
38. ISHIBASHI, N., F. YOUNG, M. NAKASHIMA, C. ABIRU, and N. HARAGUCHI. 1987. Effects of application of DD-136 on silkworm, *Bombyx mori*, predatory insect, *Agriosphodorus dohrni*, parasitoid, *Trichomalus apanteloctenus*, soil mites, and other non-target soil arthropods, with brief notes on feeding behavior and predatory pressure of soil mites, tardigrades, and predatory nematodes on DD-136 nematodes. Pp. 158–164 *in* N. Ishibashi, ed. Recent Advances in Biological Control of Insect Pests by Entomogenous Nematodes in Japan. Ministry of Education, Culture, and Science, Saga, Japan.
39. JANSSON, R. K. 1991. Biological control of *Cylas* spp. Pp. 169–201 *in* R. K. Jansson and K. V. Raman, eds. Sweet Potato Pest Management: A Global Perspective. Westview Press: Boulder, Colorado, U.S.A.
40. JANSSON, R. K., and S. H. LECRONE. 1991. Persistence of *Heterorhabditis bacteriophora* Poinar (Nematoda: Heterorhabditidae): A biological control agent of *Cylas formicarious* (Fabricius) (in press).
41. JOUVENAZ, D. P., C. S. LOFGREN, and R. W. MILLER. 1990. Steinernematid nematode drenches for control of fire ants, *Solenopsis invicta*, in Florida. Florida Entomologist 73:190–193.
42. KAMIONEK, M., H. SANDNER, and H. SERYCZYNSKA. 1974. The combined action of *Beauveria bassiana* (Bals/Vuill) (Fungi Imperfecti: Moniliales) and *Neoaplectana carpocapsae* Weiser (Nematoda: Steinernematidae). Bulletin de l'Academie Polonaise des Sciences. 22:253–257.
43. KAYA, H. K. 1985. Entomogenous nematodes for insect control in IPM systems. Pp. 283–302 *in* M. A. Hoy and D. C. Herzog, eds. Biological Control in Agricultural IPM Systems. Academic Press: New York.
44. KAYA, H. K. 1987. Constraints associated with commercialization of entomogenous nematodes. Pp. 661–664 *in* R. A. Samson, J. M. Vlak, and E. Peters, eds. Fundamental and Applied Aspects of Invertebrate Pathology. Fourth International Colloquium of Invertebrate Pathology, Wageningen, The Netherlands.
45. KAYA, H. K. 1990. Entomopathogenic nematodes in biological control of insects. Pp. 189–198 *in* R. R. Baker and P. E. Dunn, eds. New Directions in Biological Control. Liss: New York.
46. KAYA, H. K. 1990. Soil ecology. Pp. 93–115 *in* R. Gaugler and H. K. Kaya, eds. Entomopathogenic Nematodes in Biological Control. CRC Press: Boca Raton, Florida, U.S.A.
47. KAYA, H. K., and N. A. BRAYTON. 1987. Interaction between *Neoaplectana carpocapsae* and a granulosis virus of the armyworm *Pseudaletia unipuncta*. Journal of Nematology 10:350–354.
48. KLEIN, M. G. 1990. Efficacy against soil-inhabiting insect pests. Pp. 195–214 *in* R. Gaugler and H. K. Kaya, eds. Entomopathogenic Nematodes in Biological Control. CRC Press: Boca Raton, Florida, U.S.A.
49. KNIPLING, E. F. 1979. The Basic Principles of Insect Population Suppression and Management. Agricultural Handbook No. 512. U.S. Department of Agriculture, Washington, D.C. 632 pp.
50. KOBAYASHI, M., H. OKANO, and S. KIRIHARA. 1987. The toxicity of steinernematid and heterorhabditid to male mice. P. 153 *in* N. Ishibashi, ed. Recent Advances in Biological Control of Insect Pests by Entomogenous Nematodes in Japan. Ministry of Education, Saga, Japan.
51. LI, P., C. DENG, S. ZHANG, and H. YANG. 1986. Laboratory studies on the infectivity of nematode *Steinernema glaseri* to *Oncomelania jupensis*, a snail intermediate host of the blood fluke *Schistosoma japonicum*. Chinese Journal of Biological Control 2:50–53.
52. LINDEGREN, J. E., and P. V. VAIL. 1986. Susceptibility of Mediterranean fruit fly, melon fly, and oriental fruit fly (Diptera: Tephritidae) to the entomogenous nematode *Steinernema feltiae* in laboratory tests. Environmental Entomology 15:465–468.
53. LINDEGREN, J. E. 1990. Field suppression of three fruit fly species (Diptera: Tephritidae) with *Steinernema carpocapsae*. P. 223 *in* Proceedings and Abstracts of the Vth International Colloquium on Invertebrate Pathology and Microbial Control. Adelaide, Australia, August 20–24, 1990.

54. LINDEGREN, J. E., and K. A. VALERO. 1992. A simple *in vivo* method for *Steinernema feltiae* infective juvenile production and storage. Journal of Economic Entomology (in press).
55. LOCATELLI, D. P., and E. PARLEAZ. 1987. Valuatzione in laboratorio dell'attivita di *Steinernema* spp. e *Heterorhabditis* spp. su *Blatella germanica* (L.). La Difesa delle Plante 10:339–348.
56. MOLTA, N. B., and W. M. HOMINICK. 1989. Dose and time-response assessments of *Heterorhabditis heliothidis* and *Steinernema feltiae* (Nematoda: Rhabditida) against *Aedes aegypti* larvae. Entomophaga 34:485–489.
57. MOYLE, P. L., and H. K. KAYA. 1981. Dispersal and infectivity of the entomogenous nematode, *Neoaplectana carpocapsae* Weiser (Rhabditida: Steinernematidae), in sand. Journal of Nematology 13:295–300.
58. MRACEK, Z., and J. WEISER. 1983. Pathogenicity of *Neoaplectana carpocapsae* (Nematoda) for the flea, *Xenopsylla cheopis*. Journal of Invertebrate Pathology 42:133–134.
59. MULLEN, B. A., J. A. MEYER, and R. GEORGIS. 1987. Field tests of insect-parasitic nematodes (Rhabditida: Steinernematidae, Heterorhabditidae) against larvae of manure breeding flies (Diptera: Muscidae) on caged-layer facilities. Journal of Economic Entomology 80:438–442.
60. MURPHY, S. T., and D. MOORE. 1990. Biological control of the coffee berry borer, *Hypothenemus hampei* (Ferrari) (Coleoptera, Scolytidae): previous programmes and possibilities for the future. Biocontrol News and Information 2:107–117.
61. NGUYEN, K. B., and G. C. SMART, Jr. 1990. *Steinernema scapterisci* n. sp. (Rhabditida: Steinernematidae). Journal of Nematology 22:187–199.
62. NGUYEN, K. B., and G. C. SMART, Jr. 1991. Pathogenicity of *Steinernema scapterisci* to selected invertebrates. Journal of Nematology 23:7–11.
63. NUUTINEN, V., J. TYNI-JUSLIN, I. VANNINEN, and A. VAINIO. 1991. The effects of four entomopathogenic fungi and an entomopathogenic nematode on the hatching of earthworm (*Apporectodea caliginosa*) cocoons in laboratory. Journal of Invertebrate Pathology 58:147–149.
64. OBENDORF, D. L., B. PEEL, R. J. AKHURST, and L. A. MILLER. 1983. Non-susceptibility of mammals to the entomopathogenic bacterium *Xenorhabdus nematophilus*. Environmental Entomology 12:368–370.
65. OLKOWSKI, W., and S. DARR. 1989. Update: Chinese use insect-attacking nematodes against major pest. IPM Practitioner 11:1–8.
66. OSWALD, W. J. C., and D. M. MINTER. 1980. The nematode *Neoaplectana carpocapsae* as a potential biological control agent for triatomine bugs and other insects. Parasitology 80:43–44.
67. POINAR, G. O., Jr. 1979. Nematodes for biological control of insects. CRC Press: Boca Raton, Florida, U.S.A. 277 pp.
68. POINAR, G. O., Jr. 1986. Entomogenous nematodes. Pp. 95–121 *in* J. M. Franz, ed. Biological Plant and Health Protection. G. Fischer Verlag: Stuttgart, Germany.
69. POINAR, G. O., Jr. 1988. Nematode parasites of Chrysomelidae. Pp. 433–448 *in* P. Jolivet, E. Petitpierre, and T. H. Hsiao, eds. Biology of Chrysomelidae. Kluwer Academic Publishers: Dordrecht, The Netherlands.
70. POINAR, G. O., Jr. 1989. Non-insect hosts for the entomogenous rhabditid nematodes *Neoaplectana* (Steinernematidae) and *Heterorhabditis* (Heterorhabditidae). Revue de Nématologie 12:423–428.
71. POINAR, G. O., Jr. 1990. Taxonomy and biology of Steinernematidae and Heterorhabditidae. Pp. 23–61 *in* R. Gaugler and H. K. Kaya, eds. Entomopathogenic Nematodes in Biological Control. CRC Press: Boca Raton, Florida, U.S.A.
72. POINAR, G. O., Jr., and H. N. KAUL. 1982. Parasitism of the mosquito *Culex pipiens* by the nematode *Heterorhabditis bacteriophora*. Journal of Invetebrate Pathology 42:133–134.
73. POINAR, G. O., Jr., and R. GEORGIS. 1989. Biological control of social insects with nematodes. Pp. 255–269 *in* A. R. Leslie and R. L. Metcalf, eds. Integrated Pest Management for Turfgrass and Ornamentals. Environmental Protection Agency, Washington, D.C.
74. POINAR, G. O., Jr., and R. GEORGIS. 1990. Characterization and field application of *Heterorhabditis bacteriophora* strain Hp88 (Heterorhabditidae: Rhabditida). Revue de Nématologie 13:387–393.
75. POINAR, G. O., Jr., G. M. THOMAS. S. B. PRESSER, and J. L. HARDY. 1982. Inoculation of entomogenous nematodes, *Neoaplectana* and *Heterorhabditis* and their associated bacteria, *Xenorhabdus* spp. into chicks and mice. Environmental Entomology 11:137–138.
76. POINAR, G. O., Jr., G. M. THOMAS, and B. LIGHTHART. 1990. Bioassay to determine

the effect of commercial preparations of *Bacillus thuringiensis* on entomogenous Rhabditoid nematodes. Agricultural Ecosystem and Environment 30:195–202.

77. PRIOR, C. 1989. Biological pesticides for low external-input agriculture. Biocontrol News and Information 10:17–22.
78. RENN, C. 1990. A comparison of nematode containing baits with commercially available insecticidal baits for the control of adult *Musca domestica* infestation of intensive animal units. Pp. 269–274 *in* Brighton Crop Protection Conference, United Kingdom, November 19–22, 1990.
79. RING, D. R., and H. W. BROWNING. 1990. Evaluation of entomopathogenic nematodes against the Mexican rice borer (Lepidoptera: Pyralidae). Journal of Nematology 22:420–422.
80. ROVESTI, L., E. W. HEINZPETER, F. TABLIENTE, and K. V. DESEO. 1988. Compatibility of pesticides with the entomopathogenic nematode *Heterorhabditis bacteriophora* Poinar (Nematoda: Heterorhabditidae). Nematologica 34:462–476.
81. SAMISH, M., and I. GLAZER. 1991. Killing ticks with parasitic nematodes of insects. Journal of Invertebrate Pathology 58:281–282.
82. SCHROEDER, W. J. 1990. Water absorbent starch polymer: Survival aid to nematodes for control of *Diaprepes abbreviatus* (Coleoptera: Curculionidae) in citrus. Florida Entomologist 73:129–132.
83. SHETLAR, D. J. 1989. Entomogenous nematodes for control of turfgrass insects with notes on other biological control agents. Pp. 225–252 *in* A. R. Leslie and R. L. Metcalf, eds. Integrated Pest Management for Turfgrass and Ornamentals. Environmental Protection Agency, Washington, D.C.
84. SMART, G. C., Jr. 1987. Mole crickets: The worm's turn. University of Florida IFAS Research 87. 21 pp.
85. SOSA, O., Jr. 1990. Entomogenous nematodes as biological control organisms of sugarcane pests. Journal of American Society of Sugarcane Technologists 19:118.
86. SOSA, O., Jr., and J. B. BEAVERS. 1985. Entomogenous nematodes as biological control organisms for *Ligyrus subtropicus* (Coleoptera: Scarabaeidae) in sugarcane. Environmental Entomology 14:80–82.
87. SOSA, O., Jr., and D. G. HALL. 1989. Mortality of *Ligyrus subtropicus* (Coleoptera: Scarabaeidae) by entomogenous nematodes in field and laboratory trials. Journal of Economic Entomology 82:740–744.
88. TREVERROW, N., and R. BEDDING. 1990. Control of the banana weevil borer, *Cosmopolites sordidus* (Germar) with entomopathogenic nematodes. Pp. 233 *in* Proceedings and Abstracts of the Vth International Colloquium of Invertebrate Pathology and Microbial Control. Adelaide, Australia, August 20-24, 1990.
89. WANG, J. X., and Y. L. LI. 1987. Entomogenous nematode research in China. Revue de Nématologie 10:483–489.
90. WASSINK, H, and G. O. POINAR, Jr. 1984. Use of the entomogenous nematode, *Neoaplectana carpocapsae* Weiser (Steinernematidae: Rhabditida), in Latin America. Nematropica 14:97–109.
91. WEBSTER, J. M. 1980. Biocontrol: The potential of entomophilic nematodes in insect management. Journal of Nematology 12:270–277.
92. WICHT, M. C., Jr., and J. S. RODRIGUEZ. 1970. Integrated control of muscid flies in poultry houses using predator mites, selected pesticides and microbial agents. Journal of Medical Entomology 7:687–692.
93. WOUTS, W. M. 1984. Nematode parasites of lepidopterans. Pp. 655–696 *in* W. Nickle, ed. Plant and Insect Nematodes. Marcel Dekker: New York.
94. ZIMMERMAN, R. J., and W. S. CRANSHAW. 1990. Compatibility of three entomogenous nematodes (Rhabditida) in aqueous solutions of pesticides used in turfgrass maintenance. Journal of Economic Entomology 83:97–100.

Received: 5.XII.1991
Recibido:

Accepted for publication: 15.IV.1992
Aceptado para publicar: