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Entomopathogenic Nematodes: Potential For Exploration and Use in South America

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Nematóides Entomopatogênicos: Potencial Para Exploração e Uso na América do Sul

RESUMO - Nematóides entomopatogênicos apresentam potencial para o controle biológico de pragas e têm sido usados na América do Norte, Europa, Ásia e Austrália para o controle de pragas de solo e de ambientes crípticos. Esses nematóides podem ser facilmente produzidos em larga escala e ser aplicados com equipamentos convencionais. Ademais, têm ampla gama de hospedeiros e são inócuos ao ambiente. Tendo em vista história de sucesso obtido na implementação de programas de controle biológico, países da América do Sul têm grande oportunidade de desenvolver e implementar o uso de nematóides entomopatogênicos. A ênfase que tem sido recentemente dada no Brasil à produção de frutas e legumes em geral reforça a necessidade para que métodos de controle de pragas mais seguros e eficientes sejam implementados. Este trabalho apresenta uma visão geral sobre os desenvolvimentos recentes na pesquisa e comercialização de nematóides entomopatogênicos e avalia seu potencial para uso e exploração no Brasil e outros países sul-americanos.

PALAVRAS-CHAVE: Controle biológico, *Steinernema*, *Heterorhabditis*, nematóides entomopatogênicos, pragas de solo.

ABSTRACT - Entomopathogenic nematodes have potential for biological control of insect pests. They are currently used for the control of soil and cryptic pests in North America, Europe, Asia and Australia. Entomopathogenic nematodes can be easily mass-produced and applied using conventional spray equipment. They have a broad host range and are safe to the environment. Due to the history of success with implementation of biological pest control programs, South American countries have a great opportunity to develop and implement the use of entomopathogenic nematodes. Recent emphasis on fruit and vegetable production in Brazil also stresses a need to implement safer and effective pest control methods. This paper provides an overview of recent developments in entomopathogenic nematode research and commercialization, evaluates their potential for use and exploration in Brazil and other South American countries, and makes recommendations for establishing entomopathogenic nematology.

KEY WORDS: Biological control, *Heterorhabditis*, *Steinernema*, entomopathogenic nematodes, soil pests.

Brazil has a history of success with biological control projects involving the use of insect pathogenic viruses, fungi and parasitoids (Campanhola *et al*. 1995). A National Quarantine Laboratory has been established at Embrapa Meio Ambiente, Jaguariúna, São Paulo, to facilitate exchange and quarantine of biological control agents for use in national and international programs (Sá *et al*. 2000). However, the use of nematodes for pest control in Brazil is limited. The nematode *Beddingia siricidicola* (=*Deladenus siricidicola*) was introduced to control the wood wasp, *Sirex noctilio* F., in Southern Brazil (Iede *et al*. 1998). However, little has been done on entomopathogenic nematodes (Rhabditida: Steinernematidae and Heterorhabditidae), which have exceptional potential for biological control of insects especially those in the soil and in cryptic habitats (Grewal & Georgis 1998). Entomopathogenic nematodes possess a unique combination of attributes that make them a promising alternative for pest control. Pest control has traditionally relied

upon chemical pesticides, however concerns about public safety, soil and water pollution, insecticide resistance, effects on non-target organisms, and enhanced biodegradation of pesticides, have increased the pressure to shift from chemical intensive management to alternative control strategies. There are several target pests in Brazil that can be controlled with entomopathogenic nematodes. There is also a tremendous opportunity for discovery of new nematode strains and species adapted to local environmental conditions and pests. This paper provides an overview on the status of entomopathogenic nematode research and commercialization globally, reviews the research conducted in Brazil, and explores prospectus for implementation and exploration of entomopathogenic nematodes in Brazil and other South American countries.

Nematode Biology

The parasitic cycle of nematodes is initiated by the third stage infective juveniles. These non-feeding juveniles locate and invade suitable host insects through natural body openings (i.e. anus, mouth, and spiracles) or even through the cuticle when the genus *Heterorhabditis* is concerned. Once inside the host, infective juveniles invade the hemocoel and release a symbiotic bacterium, which is held in the nematode's intestine (Poinar 1990). The bacteria cause a septicemia, killing the host within 24-48h. The infective juveniles feed on the rapidly multiplying bacteria and disintegrated host tissues. About 2-3 generations of the nematode are completed within the host cadaver. When food reserves are depleted, nematode reproduction ceases and the offspring develop into resistant infective juveniles which disperse from the dead host, and are able to survive in the environment and to seek out new hosts.

The symbiotic association of entomopathogenic nematodes with specific bacteria facilitates reproduction (bacteria serve as food) and pathogenicity of the nematodes. Although axenic nematodes (nematodes without bacteria) may occasionally cause host death, they do not generally reproduce. Furthermore, bacteria alone are incapable of penetrating the alimentary tract and cannot independently gain entrance to the host's hemocoel. Thus, nematodes act as vectors to transport the bacteria into a host within which they can proliferate, and the bacteria create conditions necessary for nematode survival and reproduction within the insect cadaver. The ventricular portion of the intestine of the steinernematid infective juvenile is specifically modified for storage of symbiotic bacteria and is called an intestinal vesicle (Poinar & Leutenegger 1968, Bird & Akhurst 1983). In the infective stage of heterorhabditid nematodes, symbiotic bacteria are located in the esophagus and in the ventricular portion of the intestine (Poinar *et al*. 1977). All species of *Steinernema* are associated with bacteria of the genus *Xenorhabdus* and all *Heterorhabditis* nematode species are associated with *Photorhabdus* bacteria (Boemare *et al*. 1993). Each nematode species has a specific natural association with only one bacterial species, although any one bacterial species may be associated with more than one nematode species (Akhurst & Boemare 1990). The specificity of association between nematodes and bacteria operates on three levels (Grewal *et al*. 1997b): provision of factors that enhance infective juvenile recovery from the non-feeding stage (dauer); provision of essential nutrients for the nematode by the bacterium; and retention of the bacterium within the intestine of the non-feeding infective juvenile.

Geographic Distribution

Twenty-four species of *Steinernema* and eight of *Heterorhabditis* have been described from various insects or from the soil worldwide (Table 1). Steinernematids and heterorhabditids are ubiquitous in distribution and have been recovered from soils throughout the world (Hominick *et al*. 1996). Although some entomopathogenic nematodes have been isolated from insects naturally infected in the field, they are most commonly recovered from soil by baiting with susceptible insects (Bedding & Akhurst 1974). The wax moth larva *Galleria mellonella* (L.) is most commonly used as a 'bait'.

Host Range

The nematode-bacterium complex kills insects so rapidly that the nematodes do not form the intimate, highly adapted, host-parasite relationship characteristic of other insect-nematode associations, e.g., mermithids. This rapid mortality permits the nematodes to exploit a range of hosts that spans nearly all insect orders, a spectrum of activity well beyond that of any other microbial control agent. In laboratory tests, *S. carpocapsae* alone infected more than 250 species of insects from over 75 families in 11 orders (Poinar 1975). The nematodes attack a far wider spectrum of insects in the laboratory where host contact is assured, environmental conditions are optimal, and no ecological or behavioral barriers to infection exist (Kaya & Gaugler 1993, Gaugler *et al.* 1997). For example, foliage feeding lepidopteran larvae are highly susceptible to infection in Petri dishes, but are seldom impacted in the field, where nematodes tend to be quickly inactivated by the environmental extremes (i.e., desiccation, UV radiation, temperature) characteristic of exposed foliage. Behavioral barriers also restrict nematode efficacy to a few selected hosts or host groups (Gaugler *et al*. 1997). Some nematode species search for hosts at or near the soil surface (e.g., *S. carpocapsae* and *S. scapterisci*), whereas others are adapted to search deeper in the soil profile (e.g., *H. bacteriophora* and *S. glaseri*) (Grewal *et al*. 1994a). The former group has been referred to as "ambusher", which remains nearly sedentary while waiting for the mobile surface-dwelling hosts (Campbell & Gaugler 1993). The later group has been referred to as "cruiser" which is highly mobile, responds strongly to long-range host chemical cues, and is therefore best adapted to find sedentary hosts (Grewal *et al*. 1994a).

Mass-Production and Formulation

Entomopathogenic nematodes can be mass-produced by *in-vivo* or *in-vitro* methods. The wax moth, G. *mellonella* larvae are most commonly used to rear nematodes because

Table 1. List of valid species of *Steinernema* and *Heterorhabditis* with their original localities and sources of isolation.

Nematode species	Original locality	Original source
Heterorhabditis Poinar 1976		
H. argentinensis Stock 199*3*	Rafaela, Argentina	*Graphognathus* sp. Buchnan
H. bacteriophora Poinar 1976 = Type species	Brecon, South Australia	*Heliothis punctigera* Wallengren
H. brevicaudis Liu 1994	Fujian Province, China	Soil
H. hawaiiensis Gardener *et al*.1994	Hawaii, USA	Soil
H. indica Poinar *et al*.1992	Coimbatore, India	Soil*
H. marelata Liu & Berry 1996	Seaside, Oregon, USA	Soil
H. megidis Poinar *et al*.1987	Jeromesville, Ohio, USA	*Popillia japonica* Newman
H. zealandica Poinar 1990	Auckland, NewZealand	*Heteronychus arator* F.
Steinernema Travassos 1927		
S. abbasi Elawad *et al*.1997	Sultanate of Oman	Soil
S. affine (Bovien 1937) Wouts *et al*. 1982	Denmark	*Bibio* sp.
S. arenarium (Artyukhovsky 1967) Wouts *et al*. 1982	Central Russia	Soil
S. bicornutum Tallosi *et al*.1995	Strazilovo, Yugoslavia	Soil
S. carpocapsae (Weiser 1955) Wouts *et al*. 1982	Czechoslovakia	*Cydia pomonella* (L.)
S. caudatum Xu *et al*.1991	China	Soil
S. ceratophorum Jian *et al*.1997	Jining Province, China	Soil
S. cubanum Mracek *et al*. 1994	Western Cuba	Soil
S. feltiae (Filipjev 1934) Wouts *et al*. 1982	Denmark	*Agrotis feltiae*
S. glaseri (Steiner 1929) Wouts *et al*.1982	New Jersey, USA	*Popillia japonca* Newman
S. intermedium (Poinar 1985) Mamiya 1988	South Carolina, USA	Soil
S. karii Waturu *et al*. 1997	Central Province, Kenya	Soil
S. krausei (Steiner 1923) Travassos 1927 = Type species	Germany	*Cephaleia abietis* (L.)
S. kushidai Mamiya 1988	Hamikita, Japan	*Anomala cuprea* Hope
S. longicaudatum Shen & Wang 1991	Guangdong, China	Soil
S. monticolum Stock *et al*. 1997	Republic of Korea	Soil
S. neocurtillae Nguyen & Smart 1992	LaCrosse, Florida, USA	*Neocurtilla hexadactylla* (Perty)
S. orgonense Liu & Berry 1996	Oregon, USA	Soil
S. puertoricense Roman & Figueroa 1994	Puerto Rico	Soil
S. rarum (de Doucet 1986) Mamiya 1988	Cordoba, Argentina	Soil
S. riobrave Cabanillas *et al*. 1994	Waslaco, Texas, USA	Soil
S. ritteri de Doucet & Doucet 1990	Cordoba, Argentina	Soil
S. scapterisci Nguyen & Smart 1990	Rivera, Uruguay	*Scapteriscus vicinus* Scudder
S. siamkayai Stock *et al*.1988	Petchabun Province, Thialand	

*soil baited with *Scirpophaga excerptalis* Walker, larvae

of their commercial availability. Several researchers (Dutky *et al.* 1964, Howell 1979, Lindegren *et al*. 1993, Flanders *et al*. 1996) have described the methods of nematode infection, inoculation, and harvesting. Using the *in-vivo* process, yields between $0.5x10^5$ - $4x10^5$ infective juveniles per larva, depending on the nematode species, have been obtained. During the past few years a distinct cottage industry has emerged in the USA which utilizes the *in-vivo* process for nematode mass-production for sale, especially in the home lawn and garden markets. The *in-vivo* process, however, lacks any economy of scale; the labor, equipment, and material (insect) costs increase as a linear function of production capacity. Perhaps even more important is the lack of improved quality while increasing scale. The *in-vivo* nematode production is increasingly sensitive to biological variations and catastrophes as scale increases (Friedman 1990).

As early as 1931, Rudolf Glaser recognized the value of developing artificial culture methods for entomopathogenic nematodes and devised the first such method for *S. glaseri* (Glaser 1932). However, Glaser was unaware of the significance of symbiotic bacteria in the nutrition and pathogenicity of nematodes, which was recognized much later (Poinar & Thomas 1966). Therefore, the first successful commercial scale monoxenic culture was developed by Bedding and has come to be known as "solid" culture (Bedding 1981, 1984). In this method, nematodes are cultured

on a crumbed polyether polyurethane sponge impregnated with emulsified beef-fat and pig's kidneys along with symbiotic bacteria. Using this method approximately $6x10^5$ - $10x10^5$ infective juveniles/g of medium were achieved (Bedding 1984). Since then, this method has been commercially used in Australia, China, and USA. In a scale-up model, Friedman reported that the solid culture method is economically feasible up to a production level of approximately $10x10^{12}$ nematodes/month (Friedman 1990). Labor costs increase significantly for nematode production beyond this level, making a less expensive method of large-scale production a necessity.

Friedman also reported the development of a liquid fermentation technique for large-scale production of nematodes (Friedman 1990). In this method, costs of production decrease rapidly up to a capacity of approximately $50x10^{12}$ infective juveniles/month. This method allows consistent production of steinernematids in as large as 80,000 litter fermenters. Recent improvements in the nematode fermentation and media formulation processes have resulted in further improvements in nematode quality and yields. The current yields of *S. carpocapsae* in liquid culture average at about $2.5x10^5$ infective juveniles/g. In addition to *S. carpocapsae, S. riobravis, S. scapterisci, S. feltiae, S. glaseri, H. bacteriophora*, and *H. megidis* have been produced successfully in large-scale liquid cultures.

Several formulations have been developed for the storage and application of entomopathogenic nematodes. The shelf life of different nematode-based products varies depending on the formulation, nematode species and temperature. In the simplest type of formulation, the nematodes are impregnated onto moist carrier substrates providing substantial interstitial spaces leading to increased gas exchange. Such carriers include polyether polyurethane sponge, cedar shavings, peat, vermiculite, etc. Nematodes held on the sponge need to be hand-squeezed into water before application, whereas from the other carriers they may be applied directly to the soil as mulch. Due to the labor-intensive application, limited economy of scale, and constant refrigeration requirements, these formulations are only applicable in the small home lawn and garden markets.

During the past decade, significant progress was made in developing nematode formulations with improved shelf stability, scalability, and ease of use. The first of such formulations used activated charcoal to restrict nematode movement (Yukawa & Pitt 1985). Kaya & Nelsen (1985) were the first to report the encapsulation of entomopathogenic nematodes with calcium alginate. This discovery subsequently led to the development of a commercial nematode product, which used thin sheets of calcium alginate spread over a plastic screen to trap nematodes (Georgis 1990). For application, nematodes are released from the alginate gel matrix by dissolving it in water with the aid of sodium citrate. The alginate-based *S. carpocapsae* products were the first to possess room temperature shelf life of about 3-4 months, and le to an increased acceptability of nematodes in the high and medium value niche markets. However, the time-consuming extraction steps, and the problematic disposal of large numbers of plastic screens and containers, rendered this formulation unsuitable for large-scale use, especially in the professional turf industry.

Although nematodes have been successfully formulated in gel-forming polyacrilamides (Bedding & Butler 1994), flowable gels (Georgis & Manweiler 1994), wheat flour and attapulgite clay chips (Bedding 1988), none of these formulations offered any advantage over the alginate gels. A significant advancement was made with the advent of a water dispersible granule (WDG) formulation in which infective juveniles are encased in 10-20 mm diameter granules consisting of mixtures of various types of silica, clays, cellulose, lignin and starches (Silver *et al*. 1995). Nematode respiration declines substantially due to the introduction of anhydrobiosis in the granules, enabling extension in nematode shelf life in certain species (Grewal 2000a, b), thus offering several advantages over the existing formulations.

Application

The majority of applications involving the use of entomopathogenic nematodes are in inundative biological control. They have been most efficacious for insects in soil or cryptic habitats where there is protection from rapid desiccation and UV radiation. Major targets for entomopathogenic nematodes worldwide are listed in Table 2.

Although nematodes are generally applied as curative treatments, prophylactic applications to the soil surrounding seedlings and seeds have been advocated (see review by Grewal and Georgis 1998). Also a few attempts have been made to inoculatively release nematodes for establishment. *S. scapterisci* originally isolated from Uruguay was introduced into Florida as a classical biological control agent for the tawny mole cricket (Parkman *et al.* 1996). The nematodes were reported to have established after treatment of 50 m^2 plots in pastures with either infective juveniles or nematode-infected mole crickets.

Compatibility with Agrochemicals

Entomopathogenic nematodes are often applied to sites and ecosystems that routinely receive other inputs that may interact with nematodes including chemical pesticides, surfactants (e.g., wetting agents), fertilizers, and soil amendments. Often it is desirable to tank mix one or more inputs to save time and money. Infective juveniles are tolerant of short exposures (2-6h) to most agrochemicals including herbicides, fungicides, acaricides, and insecticides (Rovesti & Deseo 1990, Ishibashi 1993), and therefore, can often be tank-mixed. However, some pesticides can reduce nematode infectivity and survival (Grewal *et al*. 1998). Due to the continuous introduction of new active ingredients and formulations in different market segments and to differences in susceptibility of nematode species to pesticide formulations, it is difficult to provide up-to-date information. However, heterorhabditids tend to be more sensitive to physical challenges, including pesticides, than steinernematids.

Some pesticides act synergistically with entomopathogenic nematodes and therefore improve nematode

Table 2. Major target pests for entomopathogenic nematodes worldwide.

Pest group	Common name name	Life stage1	Application site	Commodity	Nematode sp.2
BLATODEA					
Blattellidae	German cockroach	A, N	Baits	Apartment buildings/structures	Sc, Hz
COLEOPTERA					
Cerambycidae	Asian longhorn beetle	L	Cryptic	Forest trees, fruit trees	Hb, Hm, Sc
	Bark beetle	L	Cryptic	Forest	Sc
Chrysomelidae	Flea beetles	L/A	Soil	Mint, potato, sweet potato, sugar beets, vegetables	Sc
	Colorado potato beetle	L	Foliage/soil	Potatoes, vegetables	Sc
	Elm leaf beetle	L	Foliage	Forest	Sc
	Striped cucumber beetle	L	Soil	Vegetables	Sc
	Rootworms	L	Soil	Corn, peanuts, vegetables	Sc, Sr
Curculionidae	Billbugs	L	Turf	Turf	Sc, Hb
	Root weevils	L	Soil, rhizomes	Banana, berries, citrus, forest seedlings, hops, mint, ornamental, sweet potato, sugar beets, vegetables	Sc, Hb, Hm, Sr
	Black vine weevil	L	Soil	Straw, ornamental	
	Rice water weevil	L	Soil	Paddy	Sc
Scarabaeidae	White grubs	L	Soil, Turf	Berries, field crops, ornamental, turf	Hb, Sg, Hm, Sk
Scolytidae	Coffee berry borer	L	Cryptic	Coffee berries	Hb
	Bark beetles	L	Cryptic	Forest	Hb, Sc
Tenebrionidae	Mealworm	L	Soil	Poultry houses	Sc
DIPTERA					
Agromyzidae	Leaf miners	L	Foliage	Ornamental, vegetables	Sc
Anthomyiidae	Cabbage maggot	L	Soil	Vegetables	Sc, Sf
Ephydridae	Shore flies	L	Soil	Ornamental, vegetables	Sf
Muscidae	Filth flies	A	Baits	Animal rearing units	Sf, Hb
Phoridae	Phorid flies	L	Compost	Mushrooms	Sc
Sciaridae	Sciarids	L	Compost	Mushrooms	Sf
	Fungus gnats	L	Soil	Ornamental, vegetables	Sf
Tephritidae	Fruit flies	L	Soil	Fruits and vegetables	Sc, Sf, Hb
Tipulidae	Crane flies	L	Soil, turf	Ornamental, turf	Sc, Hm
	House fly	A	Baits	Animal rearing units	Sf
HETEROPTERA					
Coreidae	Squash bug	A/N	Soil	Vegetables	Sc
LEPIDOPTERA					
Carposinidae	Peach borer moth	L	Soil	Apple	Sc
Cossidae	Carpenter worms	L	Cryptic	Ornamental, shrubs	Sc,
	Leopard moth	L	Cryptic	Apple, pear	Sc
Noctuidae	Cutworms,	L/P	Soil, turf	Corn, cotton,	
		armyworms			ornamental,
peanuts, turf, vegetables		Sc, Sr			
	Iris borer	L		Ornamental, vegetables,	Sc
Olethreutidae	Codling moth	L/P	Cryptic	Apple	Sc

Pterophoridae	Plume moth	L	Cryptic	Artichoke	Sc
Pyralidae	Webworms	L	Soil, turf	Cranberries, ornamental, turf	Sc
Psychidae	Bagworm	L			Sc
Sesiidae	Crown borers	L	Cryptic	Berries	Sc,Hb
	Grape root borer	L	Soil	Grapes, berries	Hb, Hz
	Peach borers	L	Cryptic	Peaches, Cherries	Sc, Hb
	Stem borer	L	Cryptic	Cucurbits, ornamental, shrubs, fruit trees	Sc, Hb
	Wood borers	L	Cryptic	Ornamental, shrubs	Sc, Hb
ORTHOPTERA					
Gryllotalpidae	Mole crickets	A/N	Turf	Turf, pastures, vegetables	Ss, Sr
Acrididae	Grasshoppers	A/N	Turf	Turf, pastures	Sc
SIPHONOPTERA					
Pulicidae	Cat fleas	L/P	Soil, turf	Pet/vet	Sc
THYSANOPTEA					
Thripidae	Western flower thrip	L	Soil	Ornamental, Vegetables	Hb, Sc, Sf
NEMATODA					
Several	Plant-parasitic nematodes	All	Soil, turf	Ornamental, turf, vegetables	Sr, Sf, Sc, Hb, Hi

1A = adult; L = larva; N = nymph; P = pupa. 2Sc = *S. carpocapsae*; Sf = *S. feltiae*; Sk = *S. kushidai*; Sr = *S. riobrave*; Ss = *S. scapterisci*; Hb = *H. bacteriophora*; Hm = *H. megidis*; Hz = *H. zealandica*.

efficacy in inundative applications (Koppenhoffer & Kaya 1998, Nishimatsu & Jackson 1998). Nematodes are also compatible with most inorganic fertilizers when applied inundatively but natural populations are negatively affected (Bednarek & Gaugler 1997).

Safety

Entomopathogenic nematodes and their associated bacterial symbionts have been proven safe to warm-blooded vertebrates, including humans (Poinar *et al*. 1982, Boemare *et al*. 1996). Cold-blooded species have been found to be susceptible to entomopathogenic nematodes under experimental conditions at very high dosages (Poinar & Thomas 1988, Kermarrec *et al*. 1991). However, under field conditions the negative results could not be reproduced (Georgis *et al*. 1991, Bathon 1996).

Practically all the possible negative impacts are limited to treated fields because of the low mobility of entomopathogenic nematodes (Downes & Griffin 1996), the cryptic environments they live in (soil, other plant growth media or tunnels inside plant material), and low survival on the foliage (Glazer 1992). Although several short-term laboratory or field studies have documented safety and or minimal adverse effects of entomopathogenic nematodes to mobile above ground non-target invertebrates (Poinar 1979, Kaya *et al*. 1982, Akhurst 1990, Georgis *et al*. 1991), their effects on soil micro fauna and flora are largely unknown. In the past five years, strong evidence has emerged that commercial applications of insect-parasitic nematodes significantly reduce populations of plant-parasitic nematodes (Smitley *et al*. 1992, Grewal *et al*. 1997a). N.P. Somasekhar, P.S. Grewal, E.A.B. De Nardo & B.R. Stinner (unpublished) found no decrease in the population of free living nematodes up to 60 days after one application of native and exotic strains of *Heterorhabditis* species in turfgrass, but a significant reduction in the population of plant-parasitic nematodes was observed.

Registration and Regulations

Currently there is no harmonized regulation related to the introduction, release and commercialization of entomopathogenic nematodes. Due to the unique dual character of the nematode-bacterium complex, they are considered macroorganisms in some countries and microorganisms in others, and therefore are regulated differently. According to the conclusions and recommendations of The Organization for Economic Cooperation and Development (OECD) and The European Comission´s Cooperation in the Field of Science and Technical Research (COST) workshop (Ehlers & Hokkanen 1996), nematodes are multicelular organisms and should not be regulated as microorganisms. However, the introduction of non-indigenous nematodes species should be regulated and the recommendations below adhere to the majority of the points included in the FAO Code of Conduct for the Import and Release of Biological Control (FAO 1996): the nematodes must be identified accurately by an accredited laboratory using either DNA analysis or well-defined morphological characters or both; specimens must be deposited in the laboratory, at least in frozen form so that the DNA can be recovered for analysis at a future date; intended target pests should be identified; prior to release the superiority in some respect to the existing entomopathogenic nematode species, as well as to other control methods, must be considered in

order to justify a release; any nematodes considered for release must have been obtained by conforming to, or in the spirit of, the Convention on Biological Diversity (United Nations 1992); details of the origin, known distribution and probable host range of the exotic entomopathogenic nematodes, and of its safety to the user, must be provided; an expert opinion based on available information of the possible impact on non-target organisms is desirable.

Introduction of Non-Native Entomopathogenic Nematodes into Brazil

The Brazilian Plant Protection Organization has adopted the FAO Code of Conduct for the Import and Release of Biological Control Agents (FAO 1996) and its similar Regional Code, established by COSAVE (South Cone Plant Protection Committee). The Brazilian National Quarantine facility plays a major role in the introduction of biocontrol agents for scientific research and must be consulted by anyone who intends to introduce exotic entomopathogenic nematodes into the country (Tambasco *et al*. 2001). Information about the importation and exportation of biocontrol agents by the quarantine laboratory can be found at http://www.bdt.org.br/bdt/biocontrol or email to lqcl-l@cnpma.embrapa.br. The introduction of a non-native natural enemy produced in another country for commercialization purpose requires registration in Brazil and the natural enemy also has to be submitted to quarantine before an Experimental Use Permit can be issued (Nardo *et al*. 1998). In Brazil, entomopathogenic nematodes are considered macroorganisms, and are exempt from registration requirements. Currently, there are no entomopathogenic nematodes commercially available in the country.

Entomopathogenic Nematodes in Brazil

The first report about entomopathogenic nematodes in Brazil dates back to the late thirties, when Pereira (1937) reported the occurrence of a new species described as *Heterorhabditis* (*Rhabditis*) *hambletoni* parasitizing *Eutinobothrus brasiliensis (*Hambl.), the cotton borer. No other reports about occurrence of entomopathogenic nematodes were published until almost 50 years later, when the presence of *Steinernema glaseri* was observed in eggs and larvae of *Migdolus fryanus* (West.), in a sugarcane area of the state of São Paulo (Pizano *et al.* 1985). Since then, soil samples baited with *G. mellonella* larvae have revealed the occurrence of *S. glaseri* in several sites in the states of São Paulo, Minas Gerais and Goiás. *Heterorhabditis* sp. has also been recently isolated from soil in the state of São Paulo. Pathogenicity studies in Brazil have been directed towards the control of *M. fryanus*, citrus and banana pests, ants and subterranean termites. Thus, laboratory and field tests were carried out in which pathogenicity of *in vivo* reared *S. glaseri* against *M. fryanus* was demonstrated only in Petri dishes (M. M. Aguillera, unpublished). Although *S. carpocapsae* in the formulation Exhibit (Biosys) has also demonstrated to be pathogenic to *M. fryanus* in the laboratory, it showed insignificant mortality in the field (Arrigoni *et al*. 1986). Reasons for negative results in the field remain to be fully understood, but they may have been due to both the nematode species and the target pest behavior or to the lack of adaptation of the exotic strain used. Schmitt *et al*. (1992) determined the efficacy of two exotic strains of *S. carpocapsae* in the field, applied as baits and soil drench for the control of *Cosmopolites sordidus* (Germar) and suggested that baiting techniques could be used for controlling adult banana weevils on a field scale. Schmitt (1993) reported isolation of two strains of *S. carpocapsae* from soil in the state of Sao Paulo. Both strains, were found to be infective to larvae and adults *C. sordidus* in laboratory and greenhouse tests. N.C. Passos, S.B. Alves & S.Silveira Neto (unpublished) obtained positive results using *S. carpocapsae* (Exhibit) to control ants [*Atta sexdens rubropilosa* (Forel)], and observed change in the ants' behavior and reduction of the amount of fungus cultured inside the nest. *Steinernema carpocapsae* caused high mortality of workers and soldiers of the termite species *Heterotermes tenuis* Hagen as reported by N.C. Passos & S.B. Alves (unpublished). Nematodes applied on corrugated cardboard used as an attractant to termites provoked repellence to the insects. L.E.B Faggin S.B. Alves & M.A. Tamai. (unpublished) also reported high mortality of soldiers and workers of that species.

A series of laboratory tests using *Steinernema* species against citrus pests was reported by J.F. Garcia & M.M. Aguillera (unpublished). Pathogenicity of *S. glaseri, S. anomali* and *Steinernema* sp. to *Parapantomorus* sp. and *Ecdytolopha aurantiana* (Lima), was demonstrated both *in vitro* and in the soil. Nematode development in dead insects was observed. These results indicate that entomopathogenic nematodes are promising biological agents that need to be studied aiming at the control of citrus pests.

The influence of organic matter on entomopathogenic nematodes was determined in an experiment carried out in the laboratory with filtercake, a residue from the sugarcane industry, added to soil inoculated with *S. glaseri*. Nematode migration was higher in substrates containing up to 50% organic matter. Addition of organic matter in rates higher than 75% interfered also on nematode persistence. It was further shown that *S. glaseri* infective juveniles migrate more towards the soil surface than to the bottom of a soil column. Nematode population decreased sharply seven days after inoculation in all treatments but infective juveniles remained infective up to 76 days (R.C.D. Rodrigues, M.M. Aguillera & N. Gobbi, unpublished).

Studies about rearing entomopathogenic nematodes *in vivo* were also carried in Brazil. Production of *S. carpocapsae* in larvae of the sugarcane borer *Diatraea saccharalis* Fab., was reported by Folegatti *et al*. (1988) and in *G. mellonella,* by Leite *et al*. (1990).

Defense mechanisms of third instar larvae of *Anastrepha fraterculus* (Wiederman) against *S. carpocapsae* and *S. glaseri*, were studied by R.F. Rodrigues-Trentini & A.T. Schmitt (unpublished) who observed that encapsulation and melanization of *S. glaseri* occurred during infection but nematodes reproduced in dead insects. Insects inoculated with *S. carpocapsae* presented only encapsulation but nematode development was delayed. Rodrigues-Trentini (1996)

reported also that in spite of defense reactions presented by *A. fraterculus*, *S. carpocapsae* and *S. glaseri* were effective against the insect. *Steinernema carpocapsae* was more efficient at 25ºC than at 15, 20 or 30ºC. Recent ultrastructure studies on the colonization process of *S. glaseri* and its symbiont bacteria in *Ecdytolopha aurantiana* larvae showed high level of multiplication of both organisms inside the insect body (J.C. Rodrigues, M.M. Aguillera & J.F.G, unpublished). Introductions of exotic strains of *S. carpocapsae* through the National Quarantine Laboratory have started to be done as it was reported by Tambasco *et al.* (1997), what indicates the raising interest of Brazilian researchers to study entomopathogenic nematodes.

Potential for Implementing Entomopathogenic Nematodes in Brazil

Brazil has several soil insect pests that are difficult to control with insecticides. Many of them have been targeted in other countries using entomopathogenic nematodes with satisfactory results. Information on the use of nematodes against selected major pests is reviewed below.

Weevils

Banana Root Weevil

C. sordidus (Coleoptera: Curculionidae) is present in almost all banana growing areas in the world, including Brazil and constitutes one of the major pests of this crop. Extensive testing of entomopathogenic nematodes (*Heterorhabditis* spp. and *Steinernema* spp.) against *C. sordidus* has occurred. Laumond *et al*. (1979) showed that *C. sordidus* was susceptible to *S. carpocapsae* (=*S. feltiae*) and this observation was confirmed in Central America (Figueroa 1990, J.E. Pena & R. Duncan, unpublished), South America (Rosales & Suarez 1998) and North America (Pena *et al*. 1993). The majority of the laboratory and field tests have been done in Australia and Tonga. Of 32 different strains and species of *Steinernema* and *Heterorhabditis* tested against the adult banana root weevil, *S. carpocapsae* BW strain was found to be most effective with over 85% of infection in laboratory (Treverrow *et al*. 1991).

C. sordidus adults are highly resistant to entomo- pathogenic nematodes due to the difficulty of nematodes to enter the host via mouth or anus. Large spiracles of the first abdominal segment offer an effective site of entry for the nematodes if they are able to pass under the tightly fitting elytra. A novel approach has been developed in which paraffin oil is added to the nematode preparation to seal the elytra. In order to respire, the beetle has to raise the elytra slightly, giving the nematodes access to the spiracles (Treverrow & Bedding 1993). Also, adult weevils are strongly attracted to holes or cuts in the rhizome or pseudostem, so a system using nematodes in traps was designed to lure and kill the attracted insects. Targeting the highly susceptible larvae instead of the more resistant adults may reduce the cost of nematode applications.

Sweet Potato Weevils

Several weevils attack root and tuber crops, among them those of the genus *Cylas* and *Euscepes* are the most important on sweet potato and cassava in Brazil and other South American countries. Mannion & Jansson (1992) assessed the virulence of ten entomopathogenic nematodes to *C. formicarius* Fairmare. Most nematodes were more virulent to larvae than to pupae. Adults were less susceptible to nematodes than other stages, and adult males were more susceptible than females. Under field conditions *S. carpocapsae* All strain, and *H. bacteriophora* HP88 strain, were shown to reduce weevil densities by 68 to 83% and 45 to 81% on plants treated with the two species, respectively (Jansson *et al.* 1990). The HP 88 strain was also found to persist in soil for a considerable time after application and a single application was as efficacious at reducing damage to storage root as two or three applications (Jansson *et al.* 1990). These results are quite interesting especially for low input cultures system, such as sweet potato and cassava in developing countries that cannot afford high investments. Kinoshita & Yamanaka (1998) tested *S. carpocapsae* against *C. formicarius* and *E. postfasciatus* (Fab.) with satisfactory results in the field.

Coffee Berry Borer

Hypothenemus hampei Ferrari (Coleoptera: Scotylidae) is a major pest of coffee seeds in Brazil and other South American countries (Waterhouse 1998). Infestations of *H. hampei* occur in coffee seeds while they are enclosed in berries on the trees and in berries that fall to the ground. Spraying of nematodes on fallen berries might remove the need to collect them, leaving them to produce mulch. Dispersal of infected adults may also spread nematodes into the pest population (Waterhouse 1998).

According to Waterhouse (1998) there appears to be only one record of nematodes attacking *H. hampei* in the field in India (Varaprasad *et al*. 1994), but in laboratory conditions, Allard & Moore (1989) showed that a *Heterorhabditis* sp. could cause high mortality of both adult and larvae and that infective juveniles were produced from adults and large larvae. Castillo & Marban-Mendoza (1996) reported differences in the infectivity of eight nematode strains to *H. hampei* larvae and found that three strains of *Heterorhabditis* sp. and one of *S. carpocapsae* caused high mortality of the larvae.

Armyworms, Cutworms and Earworms

Several species of cutworms, *Agrotis* spp. (Lepidoptera: Noctuidae), *Spodoptera frugiperda* (J.E.Smith), *S. exigua* Hübner, and *S. litorallis* Boisd. cause serious problems to agricultural, vegetable and forage crops, worldwide. Cutworms are highly susceptible to a number of entomopathogenic nematode species and strains (Morris & Converse 1991). Control of *Agrotis segetum* (Denis & Schiffermuller), with *S. feltiae* (=*N. bibionis*) in lettuce was equivalent to endosulfan (Lossbroek & Theunissen 1985), under field conditions. *A. ipsilon* has been effectively

managed with *S. carpocapsae* on golf course greens. Larvae and pupae of armyworms are very susceptible to entomopathogenic nematodes (Kaya & Grieve 1982), and can be effectively managed by nematodes. Richter & Fuxa (1990) reported 33-43% infection of *S. frugiperda* by *S. carpocapsae* in field corn. They also found that spraying of nematodes onto corn ears caused up to 71% infection of *S. frugiperda*. Molina-Ochoa *et al*. (1996) evaluated the susceptibility of *S. frugiperda* to several species of nematodes and found that the LC50 ranged from 1.5 to 20.6 and 3.4 to 37.2 nematodes/ml for larvae and prepupae, respectively. They concluded that *S. carpocapsae* All strain, *S. riobrave*, and *H. megidis* have potential for controlling *S. frugiperda*.

Corn Rootworms

The corn rootworms, *Diabrotica* spp., (Coleoptera: Chrysomelidae) are important pests of corn. In North America *D. virgifera virgifera* Leconte, and *D. barberi* (Smith & Lawrence) are the two dominant species that cause significant economic losses to maize. Nematode applications for rootworm suppression were ineffective in the early experiments (Munson & Helms 1970), but more recently in field studies, *S. carpocapsae* significantly reduced maize root damage (Ellsbury *et al*. 1996), reduced rootworm larval population (Jackson 1996), and rootworm adult emergence (Ellsbury *et al*. 1996). In some cases, nematode performance was equal to, or better than, insecticides (Wright *et al*. 1993). The limiting factors of efficacy are the need for timing of application to coincide with the phenology of susceptible stages of *Diabrotica* spp. (Jackson & Brooks 1995) and the adverse effects of desiccation on survival of the nematodes. Recent work (Nishimatsu & Jackson 1998) showed that the combined use of insecticides (tefluthrin) with entomopathogenic nematode may offer an integrated approach to increase nematode of rootworm.

Fruit Flies

Several species of fruit flies (Diptera: Tephritidae) are important pests of fruits and vegetables throughout the world. Adult flies oviposit into the fruit or vegetable and the larvae bore into the flesh of the developing fruit. Mature larvae exit the fruit and burrow into the soil to pupate surrounding the base of the host plant. Studies indicate that fruit fly larvae are highly susceptible to entomopathogenic nematodes, but the pupae and puparia are generally less susceptible (Stark & Lacey 1999, Gazit *et al*. 2000). In field trials, an average of 87% mortality of the Mediterranean fruit fly was obtained with an application of *S. carpocasae* (Mexican strain) at 500 infective juveniles/ cm^2 (Lindegren *et al*. 1990).

Potential for Exploration in Brazil

Due to the high diversity of insect species in South America and Brazil in particular, it is expected that the diversity of entomopathogenic nematodes will also be high. Further, Brazil offers a variety of ecological niches in which nematode species adapted to different environmental conditions may be found. Discovery of species and strains with greater tolerance to environmental stresses including temperature, UV, and desiccation will expand the biological control potential of entomopathogenic nematodes. Due to the availability of a range of undisturbed habitats including the savannas and the rain forest, an opportunity exists for the discovery of novel nematode species and strains in Brazil.

Explorations in Brazil for biocontrol agents are permitted to Brazilian scientists working for official institutions for scientific purposes, with no special permit required. However, a special permit is required for collections in protected areas, or when the species to be collected is threatened. Also the exportation of material collected by Brazilian people needs specific exportation permits, which are issued on request by IBAMA (Brazilian Institute of the Environment) (Moraes *et al*. 1996, Moraes & Nardo 1996). Exploration for biocontrol agents and other organisms in Brazil, by foreigners, requires a special permit, which can only be issued if the proposed work is conducted in cooperation with a Brazilian institution of recognized technical and scientific capability in the corresponding specialty. Information about exploration in Brazil for biocontrol agents and other organisms can be found at http:// www.cnpq.br/sci/exped.

The goal of any entomopathogenic nematode survey should be to capture maximum information about the species in its natural habitat. Sampling details, such as sample number, sample size, sample location, and sample depth warrant consideration when developing a protocol. Accommodation should be made to facilitate future use and interpretation of survey data by the inclusion of associated data.

Conclusions and Recommendations

We conclude that establishing entomopathogenic nematology in Brazil and other countries in South America would bring several benefits to the region. Firmly establishing insect nematology will promote the sustained use of agriculture and develop a better understanding of biodiversity. Due to the exceptional successes made with other biological control agents, Brazil is poised for developing innovative ideas to implement the use of entomopathogenic nematodes. This may be accomplished through the following: holding workshops on entomopathogenic nematodes; training scientists and students in foreign research centers; include entomopathogenic nematology as a priority in the national research programs; promote cooperative international projects involving scientists in this area that could provide training for Brazilian scientists and students; and include this area of study in university programs. National and international agreements should express the mutual interest of both parts, in terms of exchanging experiences, material and information and respecting the national and international legislation related to conservation of biodiversity and exchange of biological control agents. The development of this area in Brazil will also help other southern countries in South America, including Argentina, Chile, Paraguay and Uruguay, because many pests are common to this region.

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