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Status of entomopathogenic nematodes and their symbiotic bacteria from selected countries or regions of the world

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Abstract

Entomopathogenic nematode–bacterium complex research is being conducted in many parts of the world, and initially, a global assessment of this research was the goal of this paper. However, this was deemed impossible because there are so many researchers in many countries and regions working on these important biological control agents of soil pests. Accordingly, research activities from selected countries or regions are presented. In North America and Europe, emphasis was placed on the status of commercially available nematodes, whereas with other countries and regions, the emphasis was placed on the research activities with the nematode–bacterium complexes. The one exception was with Japan where the development of commercial nematodes was emphasized. In China, Korea, and India, research activities in the use of the nematode for controlling insect pests or soil plant pathogens was stressed. In Turkey where the research is in its initial stages, we report on the Turkish nematodes and their associated bacteria. In Central America, initial attempts to control insect pests and mass production research are reported, whereas in South America, the emphasis is on biological control of some insect pests and on some basic research with some of their native nematodes. The research is still in its early stages or non-existent in most African countries, but considerable research progress has been made in Egypt with these nematodes. Overall, the intensity of research varies by country or regions. In most cases, the research in developing countries shows that the emphasis is to demonstrate the usefulness of the entomopathogenic nematodes or their symbiotic bacteria against various pests. The ultimate goal of these research activities is to use them as biological control agents of soil pests.

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1. Introduction

Since the late 1970s, there has been a tremendous research and commercial interest in entomopathogenic nematodes (i.e., steinernematids and heterorhabditids) and their associated bacteria (*Xenorhabdus* for *Steinernema* and *Photorhabdus* for *Heterorhabditis*). This interest was sparked, in part, by (1) a lack of adequate tools to control soil-inhabiting insect pests in an effective, environmentally acceptable manner (Klein, 1990) and (2) the capability of producing these nematodes monoxenically in vitro which was followed by scale-up to commercial production levels (Ehlers, 2001). Because of the high cost of nematodes relative to chemical insecticides, these nematodes have been applied primarily against soil pests in high value crops. In addition to the biological control benefits, microbiologists found that the associated bacteria produce antibiotics and toxins and serve as model systems to understand symbiotic associations. These properties of the bacteria have generated further interest in questions about the nematode/bacterium complex (Boemare, 2002). This proliferation of research has been concentrated primarily in North America and Western Europe. In the 1980s and 1990s, Australian scientists made significant contributions in nematode mass production technology and in the understanding of the biology of the symbiotic bacteria. However, in recent years, changes in research direction and retirements have affected nematode studies in Australia.

In other parts of the world, research with these nematodes and their symbiotic bacteria is generally in the early phase and the work often was done in collaboration with scientists from developed countries. There are a few exceptions to this generalization. In this paper, we report on the research activities from selected parts of the world. For the developed countries, our emphasis is on the commercial aspects of the nematode/bacterium complex, whereas for other parts of the world, we will emphasize ongoing research activities.

Our initial concept was to be all inclusive and present a global perspective of nematode/bacterium research, especially in less developed countries. However, we were unable to cover all research activities throughout the world. For example, Vietnam (e.g., Phan et al., 2004) and Thailand have active research programs on entomopathogenic nematodes and Jordon, Azerbaijan, Ukraine, and Russia have varying degrees of research activity with these nematodes. Although we have restricted our paper to the selected countries or areas, we do recognize that many other countries and regions have made important contributions in entomopathogenic nematode–bacterium complex research. In this paper, we provide a spectrum of research activities occurring in various parts of the world.

2. North America

2.1. Background

North America was the location of the earliest in vitro mass production and field-testing of steinernematid nematodes by Rudolf Glaser and co-workers in the late 1930s (Gaugler et al., 1992) and Samuel Dutky in the 1950s (Dutky, 1959). It was not until the early 1980s that serious attempts to commercially produce nematodes were made (Friedman, 1990; Georgis, 2002). This commercial venture was spurred by the in vitro technology developed by Robin Bedding in Australia that produced large quantities of nematodes monoxenically using a solid-state culture technique (Friedman, 1990; Gaugler and Han, 2002). Bedding also developed a methodology that could harvest the nematodes relatively easily (Friedman, 1990). This sponge-based technology was a major breakthrough but was still labor intensive. To achieve economies of scale, liquid fermentation methods for nematode production were developed (Ehlers, 2001; Friedman, 1990; Gaugler and Han, 2002; Georgis, 2002). However, liquid fermentation culture was not economically feasible for the cottage industry because of the high initial capital investment required to procure and operate a bioreactor. The cottage industry continues to rely on nematodes being produced in vivo or employing the in vitro solid-state production scheme developed by Bedding (Gaugler and Han, 2002).

2.2. Commercial producers

In 2004, eight "producers" of entomopathogenic nematodes were noted in the USA and five in Europe (Table 1). (In 2005, Certis sold its nematode operation to Becker Underwood.) We define "producer" as a company that actively cultures either steinernematids or heterorhabditids for sale. In contrast, organizations that sell nematodes but are not affiliated directly with a culturer of nematodes are "distributors" are excluded from our list. However, it is not unusual for companies to assist each other in producing nematodes as this makes efficient use of a bioreactor. In addition to the North American producers, two European producers, e-nema and Koppert, have subsidiaries in North America that distribute their nematode products. A list of North American producers and distributors is located at the following website: http://www.oardc.ohio-state.edu/nematodes/nematode_suppliers.htm.

The nematode species that are commercially produced include *Steinernema carpocapsae* (Weiser), *S. feltiae* (Filipjev), *S. glaseri* (Steiner), *S. kraussei* (Steiner), *S. riobrave* Cabinillas, Poinar and Raulston, *S. scapterisci* Nguyen and Smart, *Heterorhabditis bacteriophora* Poinar, *H. indica*

Table 1
Nematode producers in Europe and the USA[a] in 2004 are listed alphabetically with no attempt to assess their relative size or sales volume

Company	Nematode species[b]	Web Site	Formulations	Markets/pests
Andermatt Biocontrol, Grossdietwil, Switzerland	Sc, Sf, Hm	www.biocontrol.ch	Clay	Greenhouse, home gardening
Asa Jung Laboratory, Oakland, California, USA	Sc, Sf	www.asajunglab.com	Clay	Varied
BioLogic Willow Hill, Pennsylvania, USA	Sc, Sf	www.biologicco.com	Bulk, dispersable granule, sponge, granular	Varied
Bionema, Umea, Sweden	Sc, Sf	www.bionema.se/	Polymer	Home gardening
Becker Underwood[c] Ames, Iowa, USA	Sc, Sf, Sg, Sk, Ss, Hm	www.beckerunderwood.com	Sprayable, dispersable granule	Greenhouse, mushrooms, mole crickets, turfgrass
Certis[d] Columbia, Maryland, USA	Sc, Sf, Sr	www.certisusa.com/aboutcertis/	Bulk, dispersable granule	Varied
e-nema GmbH, Raisdorf, Germany	Sc, Sf, Hb	www.e-nema.de	Clay, polymer	Greenhouse, tree nurseries, mushrooms, turfgrass, home gardening
Hydrogardens, Colorado Springs, Colorado, USA	Sc, H sp.	www.hydro-gardens.com	Sponge	Greenhouse
Integrated Biocontrol Greendale, Indiana, USA	Hb, Hi, Hmar	www.goodbug-shop.com/	Paste, sponge	Citrus, turfgrass, etc.
Koppert, Berkel en Rodenrijs, The Netherlands	Sf, Hb	www.koppert.nl/	Clay	Greenhouse, turfgrass
M & R Durango, Bayfield, Colorado, USA	Sc, Sf, Hb	www.goodbug.com/	Sponge	Varied
Nematec, San Ramon, California, USA	Hb	N/A[e]	Nematode wool	Nematec, San Ramon, California, USA
Owiplant, Owińska K/Poznania, Poland	Sf	www.owiplant.com.pl/	Clay	Greenhouse, mushrooms

[a] Before 2003, there was one known Canadian producer, but a recent check of the web site showed no activity in 2004.
[b] Sc, *Steinernema carpocapsae*; Sf, *S. feltiae*; Sg, *S. glaseri*; Sk, *S. kraussei*; Sr, *S. riobrave*; Ss, *S. scapterisci*; Hb, *Heterorhabditis bacteriophora*; Hi, *H. indica*; Hm, *H. megidis*; Hmar, *H. marelatus*; H sp., *Heterorhabditis* species.
[c] USA company but operates out of Littlehampton, United Kingdom.
[d] In 2005, Certis sold the nematode aspect of the company to Becker Underwood.
[e] N/A, not available.

Poinar, Karunakar and David, *H. megidis* Poinar, Jackson and Klein, *H. marelatus* Liu and Berry, and a *Heterorhabditis* species. No producer cultures all these species; each producer cultures from 1 to 6 nematode species and targets different markets (Table 1). These commercial nematodes target a number of insect pest species in high value crops which are detailed in the review paper by Georgis et al. (2006).

3. Europe

3.1. Background

Since the expansion of interest in entomopathogenic nematodes during the early 1980s, there has been a surge of research on this subject in Europe. Research has encompassed fundamental nematode biology and taxonomy, production, harvesting and formulation technology, and studies on application and efficacy.

A major contributing force for entomopathogenic nematode researchers in Europe has been the European Union (EU) sponsored COST Programme (European CoOperation in the field of Scientific and Technical Research). Although this program does not directly fund research activities, it does provide money to allow the coordination of nationally funded research on a continent wide level. This includes funding for workshops/conferences, travel costs for meetings, funding publications and short-term scientific missions for researchers to visit other participating laboratories. COST has a geographical scope beyond the EU and most of the Central and Eastern European countries are members. The present COST action including entomopathogenic nematodes, COST 850—Biocontrol Symbiosis, is comprised of representatives from Austria, Belgium, Bulgaria, Czech Republic, Denmark, Finland, France, Germany, Greece, Hungary, Ireland, Israel, Italy, Netherlands, Norway, Poland, Portugal, Spain, Sweden, Switzerland, and the United Kingdom. The regular meeting held as part of this action includes researchers from industry, government research laboratories and academia. Details of these European wide research activities and the publications from them can be found at the following website: www.cost850.ch.

3.2. Commercial producers in Western Europe

In Western Europe, entomopathogenic nematodes have been commercially produced since 1986. Currently, there are three large-scale producers of nematodes: Becker Underwood (formerly MicroBio Ltd.) is owned by a USA

company but operates out of Littlehampton, United Kingdom, e-nema is based in Germany, and Koppert has its home in The Netherlands (Table 1). These companies produce nematodes in vitro using large bioreactors. Total bioreactor volume for liquid culture production in Europe is currently at 50 m^3. In addition, there are smaller producers like Andermatt Biocontrol based in Switzerland, bionema in Sweden and Owiplant in Poland, which produce nematodes using an improved solid-state Bedding system (Gaugler and Han, 2002) (Table 1). Products are generally based on clay, vermiculite or polymer formulations. The main targeted pests for use of entomopathogenic nematodes have included larvae of sciarid flies (i.e., fungus gnats), *Bradysia* spp. and *Lycoriella* spp., in ornamentals and mushrooms, the black vine weevil, *Otiorhynchus sulcatus* (F.), in tree nurseries, ornamentals and strawberries, and grubs of the garden chafer *Phyllopertha horticola* (L.) and other grub species attacking turfgrass roots in home lawns, sports fields, and golf courses. The larval stages of these insect pests occur in the soil and can be effectively controlled by these nematodes. *Steinernema feltiae* has been the best match against sciarid larvae, whereas *Heterorhabditis* spp. have been effective against black vine weevil larvae and grubs. European researchers and producers have also begun to investigate the use of entomopathogenic nematodes to control foliar pests such as the leaf miner, *Liriomyza bryoniae* (Kaltenbach) and the western flower thrips, *Frankliniella occidentalis* (Pergande). Nematode products that are commercially available for pest suppression include *H. megidis*, *H. bacteriophora*, *S. feltiae*, *S. carpocapsae*, and *S. kraussei*.

3.3. Development of entomopathogenic nematode/bacterium research in Hungary

Hungary has been an active participant of COST, and much of the research is available through COST publications at the web site listed above (see Section 3.1). However, Hungary offers a contrast with other European countries in that its research program is still in the early phases of development. The main research foci have been to control the common cockchafer, *Melolontha melolontha* (L.), and fireblight bacterium, *Erwinia amylovora* (Burr). Both organisms are major pests of apples. In addition, some Hungarian scientists have conducted basic research on the symbiotic bacteria.

3.3.1. Bacterial isolates

Lengyel et al. (2005) have determined the taxonomic affiliation of four new *Xenorhabdus* species isolated from four *Steinernema* hosts from different countries. As compared to the five described *Xenorhabdus* species, [*X. nematophila* (Poinar and Thomas), *X. japonica* Nishimura, Hagiwara, Suzuki and Yamanaka, *X. beddingii* Akhurst, *X. bovienii* Akhurst and *X. poinarii* Akhurst (Boemare, 2002)], these isolates represented new species on the basis of 16S rRNA gene sequences riboprint patterns and physiological and metabolic properties. The new species are *Xenorhabdus budapestensis* Lengyel, Lang, Fodor, Szállás, Schumann, and Stackebrandt type strain DSM 16342 from *Steinernema bicornutum* Tallósi, Peters and Ehlers, *X. ehlersii* Lengyel et al. type strain DSM 16337, isolated from *Steinernema* sp., *X. innexi* Lengyel et al. DSM 16336 isolated from *S. scapterisci* and *X. szentirmaii* Lengyei et al. type strain DSM 16338 isolated from *S. rarum* (Doucet).

3.3.2. Application against apple pests

The grubs of *M. melolontha* can cause complete destruction of newly planted apple trees, especially in light sandy soils. The Hungarian approach has been "Integrated Fruit Production" in which the application of soil pesticides is restricted, and entomopathogenic nematodes are to be used as bioinsecticides against this insect pest. These studies have shown that these grubs are not easily controlled by entomopathogenic nematodes. However, one nematode species, *H. bacteriophora* HU86 strain, shows some promise against other white grubs and will be further explored as a possible candidate for controlling *M. melolontha*.

The fireblight bacterium is controlled by the application of streptomycin sulfate, but this antibiotic is also used for humans and its application in agriculture may lead to resistance of human bacterial pathogens to the antibiotic. Moreover, populations of *E. amylovora* have shown resistance to this antibiotic (Skold, 1995). As *Xenorhabdus* and *Photorhabdus* species produce secondary metabolites that have antibiotic activity against a number of microorganisms (Webster et al., 2002), the concept of using one or more of these metabolites to control the fireblight bacterium seems feasible. Thus, an antibiotic metabolite from a *Xenorhabdus* isolate called EMA and another from a *Xenorhabdus* isolate called EMC (Fodor et al., 2003; Máthe et al., 2003) showed promising results against the fireblight bacterium in greenhouse and field tests. Both antibiotics had cytotoxic rather than cytostatic activity. When the antibiotic from the EMA isolate was partially purified, its activity against the fireblight bacterium was comparable to streptomycin.

4. Asia

4.1. Development of entomopathogenic nematode/bacterium research in China

4.1.1. Background

During the mid-1980s and early 1990s, a comprehensive collaborative program between the Division of Entomology, CSIRO, Australia, the Guangdong Entomological Institute in Guangzhou, and the Institute of Biological Control, Chinese Academy of Sciences in Beijing was supported by the Australian Centre for International Agricultural Research (Bedding, 1990). This collaborative program trained a great number of Chinese scientists and established a research program in China, resulting in extensive publications. We can only highlight some of the accomplishments

that have been achieved with these nematodes and their symbiotic bacteria.

4.1.2. Nematode diversity

China has been a rich resource for entomopathogenic nematodes. Previously described species isolated include *S. glaseri*, *S. carpocapsae*, *H. bacteriophora* (Hominick, 2002), and *H. indica* (Fang et al., 2004). In addition, several new nematode species have been isolated and described from China including *S. caudatum* Xu et al. (1991), *S. longicaudum* Shen and Wang, *S. ceratophorum* Jian et al. (1997), and *H. brevicaudis* Liu (1994). Recently, *Steinernema guangdongense* Qiu et al. (2004) and *S. aciari* Qui et al. (2005) were described, and at least two additional *Steinernema* species are being described by Chinese scientists (Qiu et al., unpublished data).

4.1.3. Application against target pests

Two successful insect control projects with entomopathogenic nematodes, one with the peach fruit moth and the other with carpenterworms, have been reported from China. The peach fruit moth, *Carposina niponensis* Walsingham, is a serious pest of apples that causes more than $1.7 billion losses per year (Wang, 1993). *Steinernema carpocapsae* A24 strain, which was mass-produced using the solid-state culture technique developed by Bedding (1984), has been used effectively against the soil-dwelling stage of the peach fruit moth. The overwintering larvae spend nearly 8 months in the soil and in early summer, the larvae leave their hibernacula and move to the soil surface to form loose cocoons to pupate. The most appropriate time for nematode application is during this peak larval emergence from the larval hibernacula which results in reduction of the first generation of fruit moth. In field tests conducted in Shangdong Province, excellent results were obtained when the soil temperature was from 26.2 to 27.1 °C, air temperature was from 25.5 to 29.5 °C, and relative humidity was from 80 to 89% (Yang et al., 2000). Using this application strategy, fruit damage can be reduced to below 3% which is comparable to a chemical insecticide treatment (Wang, 1993; Yang et al., 2000). Recently, however, control of the peach fruit borer and other insect pests in apples is being done by wrapping each fruit to avoid damage. Although this is labor intensive, better fruit quality is obtained. Consequently, the application of *S. carpocapsae* has been substantially reduced.

The use of nematodes on the carpenterworm (Cossidae), *Holcocerus insularis* Staudinger that attacks ash, Chinese scholar tree, Chinese hawthorn, and willows, and *Zeuzera multistrigata* Moore that attacks Australian pine *Casuarina equisetifolia* L., has been highly successful (Yang et al., 1993). Both carpenterworm species bore into the tree trunk with *H. insularis* attacking older trees and several larvae often being found in the same gallery and *Z. multistrigata* attacking younger trees and only one larva occurring per tree. In addition, *Z. multistrigata* larvae will migrate from tree to tree several times before they complete their development and can cause significant damage to these trees. *Steinernema carpocapsae* has proven to be the most effective nematode species (Yang et al., 1993). The outcome from this research effort was the adoption by several cities in China to use this nematode to control street trees infested with *H. insularis*. For example, *S. carpocapsae* has been used in Tianjin, the third largest city in China, since 1987. The nematode has reduced *H. insularis* infestations from 12.6 to 4%. Other insects such as the litchi beetle, *Aristobia testudo* (Voet), can also be controlled by *S. carpocapsae* (Han et al., 1996b; Xu et al., 1995). This stem-boring insect is an important pest on litchi trees in Guangdong Province. Injection of nematodes into fresh holes made by the beetle in the twigs has resulted in 73–100% control of this insect. Other insects that may be controlled by nematodes in China are listed in Table 2.

4.1.4. Application technology

Effects of the critical parameters affecting spray application of nematodes and the addition of various adjuvants on the survival and persistence of *S. carpocapsae* on leaves of Chinese cabbage were determined (Chen et al., 2001; Jin et al., 2004). Spraying with high pressure had a negative influence on the persistence of infective juveniles (IJs) on the leaves, and the numbers of living IJs collected from the leaves significantly increased when the number of nematodes applied was <2000 IJs/ml. Generally, more IJs (10–46 nematodes/cm^2) were collected from the leaf when higher volumes of nematode suspension (3–20 ml) were applied, but their survival decreased with exposure time. The survival of the IJs on a leaf surface can be increased with the addition of antidesiccants (Chen et al., 2001; Jin et al., 2004). The number of living IJs on the leaf was 150-fold greater with 0.3% xanthan gum than when the nematodes were applied with water alone. Applying IJ suspensions with different concentrations of glycerin and with 0.5% molasses and 0.01% detergent surfactant showed similar effects.

4.1.5. Mass production

The revolving composite design was introduced to determine the effects or interactions of medium compositions (soy flour, wheat flour, yeast extract, egg yolk, lard, and water) on the growth and yield of *S. carpocapsae* A24 strain and *H. bacteriophora* H06 strain in Bedding's solid-state culture system (Han et al., 1995). Nematode yields were significantly affected by the addition of water, yeast extract, and egg yolk for *S. carpocapsae* and by addition of water and lard for *H. bacteriophora*, which indicated the importance of selecting suitable medium components and combinations for these nematode species.

Although the solid-state culture system has been very useful in nematode production for China, economies of scale are better achieved with liquid culture. Hence, a series of studies to improve nematode production using liquid culture technology were initiated. Han (1996) demonstrated that a flask containing 100 g of liquid medium yielded

Table 2
Target pests for entomopathogenic nematodes in some Asian countries

Order Family	Scientific and/or common name	Commodity	Country	Nematode sp.[a]	References
Lepidoptera					
Carposinidae	*Carposina niponensis* Walsingham	Apple	China	Sc	Wang (1993), Yang et al. (2000)
		Apple, Peach	Japan		Okazaki (unpublished)
Cossidae	*Holcocerus insularis* Staudinger	Various street trees	China	Sc	Yang et al. (1993)
	Zeuzera multistrigata Moore				
Gelechiidae	*Phthorimaea operculella* (Zeller)	Potato	India	Sb, Hi	Hussaini (2003)
Pyralidae	*Chilo suppressalis* (Walker)	Rice	Korea	Sc, Sg, Hb	Choo et al. (1991), Choo et al. (1995b)
	C. zonellus Swinhoe	Maize	India	Sc	Mathur et al. (1966), Rao et al. (1971)
		Maize	India	Sb, Hi	Hussaini (2003)
Pyralidae	*Tryporyza incertulas* (Walker)	Rice	India	Sc	Rao et al. (1971)
Pyralidae	*Glyphodes perspectalis* (Walker)	Forest	Korea	Sc	Choo et al. (1995b)
Pyralidae	*Oebia undalis* F.	Crucifers	China		Han et al. (1996a)
Plutellidae	*Plutella xylostella* (L.)	Crucifers	Korea	Sc	Somvanshi and Ganguly (unpublished)
			India	St	
			India	Sb, Hi	Hussaini (2003)
Noctuidae	*Autographa nigrisigna* (Walker)	Greenhouse	Korea	Sc	Jeon et al. (2003)
Noctuidae	*Spodoptera litura* (F.)	Vegetables	Korea	Sc	Yamanaka (unpublished)
		Strawberry	Japan	Sc	Gupta et al. (1987)
		Tobacco	India	Sf	Narayanan and Gopalakrishnan (1987)
		Tobacco	India	Sb, Hi	Hussaini (2003)
Noctuidae	*Agrotis ipsilon* (Hufnagel)	Potato	India	Sf	Singh (1993, 1997)
	A. segtum (Denis et Schiffermueller)	Potato	India	Sf	Singh (1977, 1993)
		Vegetables	Korea	Sc, Hb	Choo et al. (1988)
		Turfgrass	Korea	Sc, Sg, Hb, Sl,. Sm, H sp.	Lee et al. (1997) Kang et al. (2004)
Noctuidae	*Spodoptera depravata* (Butler)	Turfgrass	Japan	Sg, Sc	Kinoshita and Yamanaka (1998)
	Parapediasia teterrella Zincken	Turfgrass	Japan	Sg, Sc	Kinoshita and Yamanaka (1998)
	Pseudaletia separata (Walker)	Rice	India		Israel et al. (1969)
Noctuidae	*Palpita indica* (Saunder)	Vegetables	Korea	Sc, Sg, Sl, Hb,	Kim et al. (2001b)
Tortricidae	*Cydia kurokoi* (Amsel)	Chestnut	Korea	Sc, Sm, Hb	Choo et al. (2001)
Arctiidae	*Amsacta albistriga* Walker	Groundnut	India	Sc	Bhaskaran et al. (1994)
Diptera					
Anthomyiidae	*Delia antiqua* (Meigen)	*Allium* sp.	Korea	Sc	Choo et al. (1988)
Calliphoridae	*Calliphora lata* Coquillett	Outhouse	Korea	Hb	Choo et al. (1996)
Muscidae	*Muscina stabulans* (Fallén)	Outhouse	Korea	Sc, Sg, Hb	Choo et al. (1996)
	Musca domestica L.	Pig sty	China	Sf	Xu et al. (1994)
Sciaridae	*Lycoriella mali* Fitch	Mushroom	Korea	Sc, Hb	Kim et al. (2001a)
Sciaridae	*Bradysia agrestis* Sasakawa	Greenhouse	Korea	Sc, Sg, Sl, Hb, H sp.	Kim et al. (2003a) Kim et al. (2004)
Coleoptera					
Curculionidae	*Aristobia testudo* (Voet)	Litchi	China	Sc	Xu et al. (1995), Han et al. (1996b)
Curculionidae	*Psacothea hilaris* (Pascoe)	Fig	Japan	Sc	Tsutsumi and Yamada (1995)
Curculionidae	*Curculio sikkimensis* (Heller)	Chestnut	Korea	Sm, Hb	Choo et al. (1995b)
Curculionidae	*Cylas formicarius* (Summers) *Euscepes postfasciatus* (Fairmaire)	Sweet potato	Japan	Sc	Yamaguchi and Kawazoe (1997)
Curculionidae	*Otiorhynchus sulcatus* (F.)	Nursery	Japan	Sc	Yamanaka (unpublished)
Curculionidae	*Sphenophorus venatus* Chittenden	Turfgrass	Japan	Sc	Yamaguchi and Kawazoe (1997)
Curculionidae	*Rhynchophorus ferrugineus* Olivier	Palm	Japan	Sc	Iiboshi and Izono (2003)
Curculionidae	*Dichocrocis punctiferalis* (Guenée)	Chestnut	Korea	Sm, Hb	Choo et al. (1995b)
Curculionidae	*Agelastica coerulea* Baly	Forest	Korea	Sc	Choo et al. (1991)
Curculionidae	*Lasioderma serricorne* Fitch	Brinjal (egg plant)	India	Sb, Hi	Hussaini (2003)
Curculionidae	*Odoiporus longicollis* Oliver	Banana	India	Sb, Hi	Hussaini (2003)
			China	Sc	Peng and Han (1996)
Curculionidae	*Rhynchites foveipennis* Frm.	Pear	China	Sc	Liu et al. (1991)
Chrysomelidae	*Phyllotreta striolata* (F.)	Vegetables	China	Sc	Peng and Han (1996)
Scarabaeidae	*Exomala orientalis* (Waterhouse)	Turfgrass	Korea	Sc, Sg, Hb	Choo et al. (2002a)
Scarabaeidae	*Adoretus tenuimaculatus* Waterhouse	Turfgrass	Korea	Sg, Hb	Lee et al. (2002b)
Scarabaeidae	*Ectinohoplia rufipes* (Motschulsky)	Turfgrass	Korea	Sc, Sg	Choo et al. (2002a)
Scarabaeidae	*Anomala* sp.	Vegetables	India	Sc	Rajeswari et al. (1984)
		Turfgrass	Japan	Sg	Yamanaka et al. (1995)

Table 2 (*continued*)

Order Family	Scientific and/or common name	Commodity	Country	Nematode sp.[a]	References
Thysanoptera					
Thripidae	*Frankiniella occidentalis* (Pergande)	Greenhouse	Korea	Sc, Hb	Jeon et al. (2003) Kim et al. (2003b)

[a] Sb, *Steinernema bicornutum*; Sc, *S. carpocapsae*; Sf, *S. feltiae*; Sg, *S. glaseri*; Sl, *S. longicaudum;* Sm, *S. monticolum*; St, *S. thermophilum;* Hb, *H. bacteriophora*; Hi, *H. indica*; H sp., *Heterorhabditis* species.

3.2×10^7 IJs of *S. carpocapsae* in 16 days using an inoculum of 8×10^5 IJs and 3×10^7 IJs of *H. bacteriophora* in 12 days with an inoculum of 5.6×10^6 juveniles. Further studies on the effects of inoculum size and culture time on *S. carpocapsae* and *H. bacteriophora* yields showed that IJ production was positively influenced by inoculum size ranging from 4.6 nematodes/g to 4.6×10^4 nematodes/g of medium for *S. carpocapsae* and 5.5 nematodes/g to 5.5×10^4 nematodes/g of medium for *H. bacteriophora* and culture time of nematodes ranging from 8 to 24 days for *S. carpocapsae* and 10–30 days for *H. bacteriophora*.

Effects of various dry flours and other components on the growth and yield of *S. carpocapsae* were established by a rotating composite design in a liquid culture system with 100 g medium combinations in 500-ml flasks. Nematode inocula were introduced into the medium 2 days after inoculation with phase I symbiotic bacterium. The highest yield of 4.3×10^5 IJs/g was obtained with an inoculum of 5×10^3 IJs/g in 16 days (Han et al., 1998).

4.1.6. Bacterial metabolites

In recent years, the metabolites of *X. nematophila* have been tested against several plant-pathogenic *Phytophthora* species. Much of the research has focused on the fermentation filtrate (metabolites minus the bacterial cells) to inhibit growth of *Phytophthora boehmeriae* Sawada, the causal agent of boll rot of cotton, *P. infestans* (Mont.) de Bary, the causal agent of potato late blight, and *P. sojae* Kaufmann and Gerdemann, the causal agent of soybean late bright. The metabolites of eight strains of *Xenorhabdus* were tested against *P. boehmeriae* in the laboratory (Yang et al., 1998). The result showed that different strains have different antibiotic effects against *P. boehmeriae* when the fermentation filtrate was diluted. For example, when the filtrate was diluted five times, 100% hyphal growth inhibition occurred with all *Xenorhabdus* strains. When the fermentation filtrate was diluted 10 and 15 times, 88–95% and 50–80% inhibition, respectively, was observed. The most active strains against *P. boehmeriae* were NC513 and NC32, followed by A24. The hyphae showed an increase in abnormal branching, hypertrophy of the hyphal tip followed by cell breakage, and leakage of the cytoplasm into the substrate. For *P. infestans*, a concentration of 1.5–3 and 6–59 ml/L of the fermentation filtrate inhibited hyphal growth rate by 70% and 90–100%, respectively (Yang et al., 2001). Although the hyphal growth was inhibited, the hyphae were not completely inactivated at 12–50 ml/L, but sporangium germination was reduced by 50% at 50 ml/L. For *P. sojae*, the hyphal growth rate was reduced by 69% at 6 ml/L and inhibited by 100% at 50 ml/L of the fermentation filtrate (Yang et al., 2002). Sporangia formation was inhibited by 97–99% at 10–100 ml/L of the fermentation filtrate.

When potato tubers were soaked in the fermentation filtrate, the metabolites permeated up to 3 cm through the tuber tissue within 24 h, and this approach shows potential for the control of potato late blight (Yang et al., 2001). In potted plant tests, disease reduction reached 70 and 76% at 25 and 50 ml/L of the fermentation filtrate, respectively, 7 days after inoculation with the blight organism. With *P. sojae*, soybean seeds were soaked in 50 ml/L of the fermentation filtrate for 6 h prior to planting resulting in 80% disease reduction 9 days post-planting (Yang et al., 2002). However, this effect declined to 34% at 12 days post-planting. When soil infested with sporangia was treated with 50 ml/L of fermentation filtrate or 1.7 ml/L of the fungicide, mezineb, there was a 76 and 84% disease reduction, respectively.

Inhibitory activity of the by-products of 38 strains of *Xenorhabdus* was tested against the rice pathogenic fungi, *Rhizoctonia solani* Kühn and *Pyricularia grisea* Sacc. (Xin et al., 2004). The fermentation filtrate showed high inhibitory activities against mycelial growth and sclerotium and conidial germination of both fungal pathogens. In particular, YNa111 and YNd173 strains showed strong inhibitory activity against the growth of both pathogens in vitro. The relative inhibitory rate of YNa111 against *R. solani* was 100 and 82% at 40 and 160 dilutions of the filtrate, respectively, whereas that of YNd173 against *P. grisea* was 88 and 76% at 5 and 10 dilutions, respectively. These studies of the use of metabolites against the various plant pathogens demonstrate the potential of using them to control plant diseases in agricultural systems.

The insecticidal activity of 28 strains of *Xenorhabdus* and *Photorhabdus* to *Helicoverpa armigera* (Hübner) and *Pieris rapae* (L.) were studied. The culture broths of four strains from *X. nematophila* resulted in high anti-feeding activity to both neonate and 3rd instar larvae of *H. armigera* as well as causing significant mortality. The mortalities of neonate larvae and the rates of growth inhibition of 3rd instar larvae of *H. armigera*, ranged from 88 to 98% and from 94 to 96%, respectively. CB1 and CB152 strains of *Photorhabdus luminescens* (Thomas and Poinar), CB19 strain of *X. nematophila* and CB32 strain of *X. poinarii* had better anti-feeding activity against*P. rapae* larvae (Liu et al., 2003).

Xenorhabdus CB6 from *S. carpocapsae* isolated from an orchard in Beijing had high antibiotic and insecticidal activity. The morphological, physiological, and biochemical characteristics of CB6 were the same as other *X. nematophila* isolates, and the sequence of 16S rRNA fragments showed 99% homologous among strain CB6 and other strains of *X. nematophila*. Strain CB6 clustered together with four other strains of *X. nematophila* in a phylogenetic tree and was named *X. nematophila* var. *pekingensis* (Pang et al., 2004). In addition, the effect of CB6 culture broth on mortality and development of *Plutella xylostella* (L.) was studied. Insect larvae fed leaves treated with fermentation liquid for 3 days resulted in 85, 68, and 59% mortality of 1st, 2nd, and 3rd instar larvae, respectively. The 3rd instar larvae feeding on treated leaves for 48 h showed nearly 100% growth inhibition, the pupation rate was 16%, and the adults could not emerge completely from the pupal stage (Li et al., 2003).

A cosmid library was constructed with *X. nematophila* BP strain, and two clones of the resulting library, cos76 and cos83, were found to have oral toxicity against neonates of the cotton bollworm, *H. armigera* (Liu et al., 2003). To identify the toxin genes presented in these two clones, seven pairs of primers were designed based on published sequences, and used for polymerase chain reaction (PCR) amplification from both clones. All seven pair primers yielded products from cos83, whereas only five were obtained from cos76. All PCR products were sequenced, and each sequence of the five PCR products obtained from cos76 was identical with that from cos83, indicating that toxin genes in cos76 are part of those in cos83. Basic local alignment search tool (BLAST) results showed that the amino acid sequences deduced from seven PCR products from cos83 have a mean homology of 95 and 65% with those from the insecticidal toxin genes of *X. nematophila* PMF1296 strain and *P. luminescens* W14 strain, respectively. Thus, the toxin produced by cos83 is a member of the oral toxin family of the symbiotic bacteria but they differ substantially from other members of the family (Cui et al., 2003).

4.2. Development of entomopathogenic nematode/bacterium research in India

4.2.1. Background

Much of the research conducted in India has been reviewed by Rahman et al. (2000). Most of the early work focused on the biological control potential of these nematodes, especially with *S. carpocapsae*. For example, Rao and Manjunath (1966) suggested that the DD-136 strain of *S. carpocapsae* could be useful in the control of insect pests of rice, sugarcane and apple. Table 2 includes some of the insect pest species that could be controlled with entomopathogenic nematodes. In a number of other cases, insects were susceptible to the nematodes, but whether they could be economically controlled feasibly remains to be seen. Although efficacy research has been conducted in India with these nematodes since the mid-1960s, research programs dealing with other aspects of entomopathogenic nematodes started in earnest only in 1998 at the Indian Agricultural Research Institute in New Delhi. Besides the New Delhi facility, other laboratories that focus on entomopathogenic nematodes include Project Directorate of Biological Control (PDBC, Bangalore), Gujarat Agricultural University (GAU), Anand and Tamil Nadu Agricultural University (TNAU), Coimbatore.

4.2.2. Nematode diversity

The initial research with entomopathogenic nematodes in India was conducted primarily with exotic species/strains of *S. carpocapsae*, *S. glaseri*, *S. feltiae*, and *H. bacteriophora* imported by researchers. In many cases, these nematodes yielded inconsistent results in field trials, probably due to their poor adaptability to the local agro-climatic conditions. India, as is the case with many other parts of the world, has a rich biodiversity resource because of its varied geographic, climatic, and weather conditions. It is divided into 15 agro-climatic and agro-ecological zones, which for the most part consist of tropical and subtropical areas. Therefore, a search for indigenous species/strains resulted in a number of nematode isolates from different parts of India (Ganguly, 2003).

Among the indigenous nematode isolates, two have been described as new species, *H. indica* (Poinar et al., 1992) from Tamil Nadu and *S. thermophilum* Ganguly and Singh (Ganguly and Singh, 2000, 2003) from New Delhi. Other species identified as indigenous isolates include *S. carpocapsae* (Hussaini et al., 2001), *S. bicornutum* (Hussaini et al., 2001), *S. riobrave* (Ganguly et al., 2002), *S. feltiae* (Ganguly and Sosamma, unpublished data) and *H. bacteriophora* (Sivakumar et al., 1989). Hussaini et al. (2001) also identified some of the native populations of *Steinernema* by restriction fragment length polymorphism (RFLP) analysis and analysis of the PCR-amplified ITS-rDNA region using 17 restriction enzymes. These results showed that *S. abbasi* Elawad, Ahmad and Reid, and *S. tami* Luc, Nguyen, Reid and Spiridonov were present in India. The genetic relatedness among the native *Steinernema* species was studied using 11 decamer oligonucleotide primers to generate random amplified polymorphic DNA (RAPD) fragments. Based on the RAPD profiles, *S. thermophilum* was differentiated from other native strains of *Steinernema* (Umarao et al., 2002).

In addition, surveys have revealed natural occurrence of several species/strains of *Steinernema* and *Heterorhabditis* in Andeman and Nicobar islands (Prasad et al., 2001), Gujarat (Vyas, 2003), Kerala (Banu et al., 1998), New Delhi (Ganguly and Singh, 2000), and Tamil Nadu (Bhaskaran et al., 1994). Some of these remain to be identified. For instance, 15 entomopathogenic nematodes were isolated from Central and North Gujarat for management of *Helicoverpa armigera* (11 *Steinernema* spp. and four *Heterorhabditis* spp.). Among these isolates, EPN-3 (Anand) and EPN-16 (Dehgam) were highly virulent to *H. armigera* with

96.8 and 70.9% larval mortality, respectively, at the rate of 2000 IJs/5 chickpea plants/pot (Vyas, 2003; Vyas et al., 2002).

4.2.3. Studies on the symbiotic bacterium of S. thermophilum

The symbiotic bacterium associated with *S. thermophilum* was isolated from its IJs in Luria–Bertoni broth medium. Molecular analysis of this bacterium as well as three other known species (*Xenorhabdus nematophila* from *S. carpocapsae*, *X. poinarii* from *S. glaseri* and *X. beddingii* from an Australian strain of *Steinernema*) was undertaken. Comparison of the RFLP profiles of PCR-amplified 16S ribosomal DNA using the restriction enzymes *Hae*III and *Msp*I, revealed a characteristic banding pattern that differentiated the bacterium associated with *S. thermophilum* from the other three species of *Xenorhabdus* used for the comparative analysis (Ganguly, unpublished data).

4.2.4. Ecological characterization of S. thermophilum

Much of the research on entomopathogenic nematodes in India has focused on assessing susceptible hosts for biological control programs. Recently, more basic studies have been initiated on these nematodes. For example, a study on the ecological characterization of *S. thermophilum* was conducted and included its host range, optimum temperature and moisture requirements, and foraging behavior (Ganguly and Singh, 2001).

The host range study showed that the IJs of *S. thermophilum* infected and reproduced on many different orders of insects (Ganguly and Gavas, 2004b). These included seven species in Lepidoptera, four in Orthoptera, and one each in Hemiptera, Isoptera, and Coleoptera. The higher nematode inoculum level (500 IJs/insect) resulted in higher insect mortality and nematode development compared with the lower inoculum level (50 IJs/insect). A terrestrial pillbug (crustacean) was also susceptible to the nematode, but two pollinating hymenopteran insects (rock bee and Indian bee) and two blattodean species (American and German cockroach) were not susceptible to infection.

Steinernema thermophilum infected greater wax moth larvae at a temperature range from 10 to 35 °C and reproduced from 20 to 35 °C. The time for first emergence of IJs was 25, 7, 6, and 5 days after inoculation at 20, 25, 30, and 35 °C, respectively. Recent studies on tolerance at higher temperature showed that the survival of the IJs in aqueous suspensions at 40 °C was 100, 80, 60, 40, 30, 20, and 10% after 1, 2, 3, 4, 5, 6, and 7 h of incubation. The LT_{50} at 40 °C was 3.5 h. The IJs could find and infect the host at soil moisture levels varying from 1 to 19% (w/w). Insect mortality increased significantly at 3% moisture and mortality gradually increased up to the 9% moisture level, and thereafter, it declined. Based on these results, the optimum temperature range for infection and reproduction of *S. thermophilum* was 25–35 °C, and the optimum moisture level was 9% soil moisture with and optimal range from 3 to 16% (Ganguly and Gavas, 2004a).

The foraging behavior of *S. thermophilum* was conducted at various soil depths (0, 2, 5, and 10 cm). These studies revealed that the IJs could find their host at the soil surface as well as down to 10 cm. The number of IJs that penetrated and established in the insect hemocoel showed no significant difference among the various depths studied. It indicated that *S. thermophilum* adopts an intermediate (cruising and ambushing) foraging strategy and may be an ideal biological control agent against insect larvae and their pupae located at varying depths in the field.

Efficacy of *S. thermophilum* was tested against diamondback moth, *P. xylostella*, infesting cabbage under field conditions (Ganguly and Somvanshi, unpublished data), using a liquid-based formulation at three concentrations (1000, 2000, and 3000 IJs/ml) as foliar spray. *Steinernema thermophilum* caused 37–45% mortality of diamondback moth larvae on cabbage, even when the minimum temperature was 5 °C.

4.2.5. Bacterial metabolites

Cell-free culture (i.e., fermentation) filtrates of *Xenorhabdus* and *Photorhabdus* spp. were tested for their toxicity against the larvae of *H. armigera*, *Phthorimaea operculella* (Zeller), *P. xylostella* and *Spodoptera litura* Fabr. Larval mortality of the insects was higher with the fermentation filtrates than with the bacterial cells. Both the cells and fermentation filtrates of *Photorhabdus* caused higher larval mortality than *Xenorhabdus*. Larvae of *P. xylostella* and *P. operculella* had 100% mortality at 48 h after exposure to the fermentation filtrates, whereas larvae of *H. armigera* and *S. litura* had 58 and 49%, respectively. Late 3rd instars of *H. armigera* and *S. litura* experienced lower mortality than the 2nd instars when exposed to the fermentation filtrates (Nagesh et al., 2003).

4.2.6. In vitro production

Eight artificial media have been assessed for entomopathogenic nematode production. These media were (1) chicken-offal, (2) egg yolk, soy flour, and cholesterol, (3) Wout's medium, (4) wheat flour, (5) dog food, (6) modified dog food medium, (7) modified wheat flour, and (8) modified egg yolk. Wout's medium, modified egg yolk, soy flour, and cholesterol and modified dog biscuit media yielded the highest number of IJs of *S. carpocapsae*, *S. tami*, and *H. indica*. The yield varied from 5.6×10^5 to 6.9×10^6 IJs/250 ml flask. However, these media did not support production of *S. bicornutum* and *S. abbasi* (Hussaini, 2003).

4.2.7. Formulations and commercialization

Comparison of three (talc-, alginate-, and water-based) formulations of entomopathogenic nematodes revealed better survival of IJs in water formulations than the talc- and alginate-based for *Steinernema* sp. (Hussaini, 2003). In comparison, *H. indica* survived better in talc-based formulation than in the other formulations.

In the 1980s, one company (Ecomax) produced the non-native *S. carpocapsae* and *H. bacteriophora* under the trade names, Green Commandos and Soil Commandos, respec-

tively. However, these nematodes were not efficacious against insects under field conditions probably because of their poor adaptability to environmental conditions in India or production or formulation problems. These nematodes were withdrawn from the market, and presently no company is marketing entomopathogenic nematodes in India.

4.3. Development of entomopathogenic nematode/bacterium research in Japan

4.3.1. Background

During the early 1980s, the study of entomopathogenic nematodes was introduced by Professor N. Ishibashi, Saga University, Saga. He organized Japanese researchers from government and university laboratories and commercial companies to conduct basic and applied research. Much of the research has been published in two books edited by Ishibashi (1987, 1990).

In 1984, SDS Biotech K.K., initiated research on entomopathogenic nematodes for commercialization in Japan. In 1993, *S. carpocapsae*, the first nematode, was registered in Japan. This nematode was initially produced in the USA and shipped to Japan and sold under the trade name Biosafe. In 2000, the company initiated production technology research with *S. glaseri* under a grant from the Japanese government and obtained the registration of a *S. glaseri*-based product under the trade name Biotopia. Currently, *S. carpocapsae* and *S. glaseri* are being produced in liquid culture by a company in UK and shipped to Japan for use.

Another nematode, *S. kushidai* Mamiya, that shows host specificity to white grubs, was discovered in Japan (Mamiya, 1988) and eventually commercialized in 1997 under the trade name Shibaichi-Nema by Kubota Corporation, Tsukuba. However, for a variety of reasons including poor timing for sale of biological products because of the depressed Japanese economy, inconsistent production in vitro, and the availability of new, effective chemical pesticides for golf course management this product was removed from the market.

4.3.2. Application against target pests

Steinernema carpocapsae was introduced to control the hunting billbug and noctuid larvae in turfgrass (Table 2). It proved to be a very effective nematode against the hunting billbug, an insect probably introduced from the USA, that was spreading to most Japanese golf courses after its initial discovery in 1986 (Kinoshita and Yamanaka, 1998). However, S. *carpocapsae* was ineffective against white grubs, and a nematode that offered better control against these pests was needed. Consequently, this led to the decision to develop *S. glaseri* for use against white grubs and to complement *S. carpocapsae* (Biosafe) in management of turfgrass pests (Yamanaka et al., 1995).

With the establishment of a nematode market in turfgrass, the next phase was to expand the use of *S. carpocapsae* beyond the turfgrass market (Table 2). The following insects received the highest priority because they were susceptible to nematodes and, in some situations, the nematodes offered the most logical alternative control tactic. Accordingly, this nematode has been applied in agricultural and horticultural systems as possible control agents for the common cutworm, *Spodoptera litura* in strawberries, yellow spotted longicorn beetle, *Psacothea hilaris* Pascoe in figs and the red palm weevil, *Rhynchophorus ferrugineus* Olivier on palms. It is also being used to augment other control tactics against the sweetpotato weevil, *Cylas formicarius* (Summers), and the West Indian sweetpotato weevil, *Euscepes postfasciatus* (Fairmaire) in a National Eradication Project (Yamaguchi and Kawazoe, 1997).

In strawberry cultivation, drip irrigation placed under multi-film plastic is an efficient system to produce this crop. In many instances, the strawberry growers apply *Bacillus thuringiensis* Berliner and insect growth regulators for suppression of the early larval stages of the cutworm, but older larvae are hidden under the plastic and are less susceptible to the selective insecticides. On the other hand, *S. carpocapsae* is effective against the older cutworms and can be applied through the irrigation system (Yamanaka, unpublished data). Similarly, figs, a high value crop in Japan, are attacked by borers, especially the yellow spotted longicorn beetle, that invade the tree trunks or main branches and weaken the trees. Chemical insecticides effectively control the younger borer larvae whereas older larvae occur deeper in the wood and are more difficult to kill. *Steinernema carpocapsae* applied as a spray or with a paintbrush to the gallery opening has given high efficacy (Tsutsumi and Yamada, 1995).

Okinawa and the southwest islands of Japan have unique insect pests because of the subtropical climate. Recently, the government initiated a control project against the sweetpotato weevil and the West Indian sweetpotato weevil. These insects infest sweet potatoes as well as many other plant species in the genus *Ipomoea*. These latter plants grow widely near the seashore and forest where the use of chemical insecticides is restricted. As a result, *S. carpocapsae* which has shown high activity against adult weevils is being used in these habitats (Yamaguchi and Kawazoe, 1997). In the case of the peach fruit moth (*Carposina niponensis*) in apple, peach, and other fruit trees, part of the life cycle occurs in the soil where *S. carpocapsae* has provided good efficacy (see Section 4.1.3). Integrating the nematode with a pheromone treatment appears to be a feasible approach for reducing damage to apples and decreasing application of chemical insecticides (Okazaki, unpublished data). In June, 2004, Biosafe was officially approved for use against this insect.

Future label expansions of *S. carpocapsae*-based products include control of the red palm weevil and leaf rollers in apples. The red palm weevil invaded Okinawa in 1975 and was not a major problem until it invaded Kyushu in the late 1990s where palms are planted along highways. Some of these palms are 30–40 years old and are being killed by the weevils. Applications of *S. carpocapsae* on the

top of palms in 2003 showed promising results against this insect (Iiboshi and Izono, 2003).

4.4. Development of entomopathogenic nematode/bacterium research in Korea

4.4.1. Background and nematode diversity

Entomopathogenic nematode research in Korea was initiated during the mid-1980s. Initial studies of entomopathogenic nematodes were laboratory-based with *S. carpocapsae* All strain and *H. bacteriophora* NC1 strain, both of which were isolated in the USA (Poinar, 1990). Subsequently, surveys were conducted in Korea that demonstrated the biodiversity of entomopathogenic nematodes with more than 30 isolates found in soil from forests, riparian habitats, agricultural fields, golf courses, and seashores and from insect cadavers (Choo et al., 1995a; Lee et al., 1996a,b; Stock et al., 1997a,b, 2001). From these isolates, a number of species were identified including *S. carpocapsae*, *S. glaseri*, *S. longicaudum*, *H. bacteriophora*, and *H. megidis* (Choo et al., 1995a; Stock et al., 1997a,b). One isolate, *S. monticolum* Stock, Choo and Kaya, was described as a new species (Stock et al., 1997b). Many of these Korean isolates have been evaluated against important insect pests of rice, vegetables, forest, turfgrass on golf courses, and crops in greenhouses.

4.4.2. Ecological characterization

Ecological characterization studies done in the laboratory such as the effects of temperature and moisture, survival, storage, reproduction, establishment, and behavior of some Korean nematodes isolates have been made. Most of these studies involved *S. carpocapsae*, *S. longicaudum*, *H. bacteriophora*, and an unidentified *Heterorhabditis* sp. (Choo et al., 1999, 2002b, Chung, 2001). For example, these studies showed that 24 °C was the optimal temperature for infection and reproduction for these species, whereas 30 °C and <13 °C were unfavorable for infection and reproduction (Choo et al., 1999, 2002b). In addition, soil texture and presence of roots affected host finding of *S. carpocapsae* and *H. bacteriophora*. In organic soil containing plant roots, the percentage of hosts infected was significantly greater than in humus with no roots. In a sand-loam-clay soil, the percentage of host infected was not affected by the presence of roots (Choo and Kaya, 1991). In field studies, Choo et al. (2002b) and Kim et al. (2003a) demonstrated that entomopathogenic nematodes persisted sufficiently in the field to obtain some degree of pest suppression.

4.4.3. Application against target pests

The nematode/bacterium complex has been tested against a number of Korean insect pests (Table 2). Some notable successes have been demonstrated in the greenhouse and on golf courses. One of the major sources of seedling transplants for farmers comes from the greenhouse industry (Kim et al., 2004; Park et al., 1999). In the early 1990s, the fungus gnat, *Bradysia agrestis* Sasakawa (recently identified as *B. difformis* Frey), was found infesting greenhouses and became one of the main pests of greenhouse crops (Park et al., 1999). This insect damages roots and stems of cucumber, eggplant, squash, gerbera, lettuce, lily, carnation, melon, and watermelon in greenhouses year round (Kim et al., 2000). In tests conducted on watermelon seedlings, *S. carpocapsae* was effective against all stages of *B. agrestis* except for the egg and first instar (Kim et al., 2004). When the watermelon seed was treated with *S. carpocapsae* at sowing, the larval and adult density of *B. agrestis* was significantly reduced compared to the control. Moreover, watermelon seedling death was 25.5–71.6% in the control plots compared with 0.2–1.9% and 0.1–1.2% in nematode-treated plots at the 17th and 34th day post-treatment, respectively. *S. carpocapsae*, *S. glaseri*, *S. longicaudum*, *H. bacteriophora*, and an unidentified *Heterorhabditis* sp. were also effective against *B. agrestis* and persisted during the 35-day-period the watermelon seedlings were in the greenhouse and for one week after transplanting to the field (Kim et al., 2003a).

Korean golf courses are infested with several species of white grubs including *Exomala orientalis* (Waterhouse), *Adoretus tenuimaculatus* Waterhouse, and *Ectinohoplia rufipes* (Motschulsky). Choo et al. (2002a) demonstrated that *E. rufipes* larval mortality was 70.2–78% in the *S. carpocapsae* and *S. glaseri* treatments compared with 15.7% in the control. *Exomala orientalis* larvae were also significantly reduced in the *S. carpocapsae*, *S. glaseri*, and *H. bacteriophora* treatments, but the efficacies were variable depending on nematode species/strain, golf courses, and year (Choo et al., 2002a). For example, with *E. orientalis* larvae, 83, 63, and 72% reduction was observed with *S. carpocapsae*. All strain, *S. glaseri* Hanrim strain, and *H. bacteriophora* Hamyang strain, respectively, in the first year test, but 90 and 98% reduction was observed with *S. glaseri* Dongrae strain and *H. bacteriophora* Hamyang strain, respectively, at the same golf course in the second year (Choo et al., 2002a). A similar trend was shown with *A. tenuimaculatus* where 56.3% reduction was recorded with *H. bacteriophora* Jeju strain, 40.8% by an unidentified *Heterorhabditis* sp., and 28% by *S. carpocapsae* Pocheon strain compared with 6.8% in the control (Lee et al., 2002b).

Because registered chemical insecticides and some nematode species were not sufficiently effective against some white grubs, combinations of control tactics have been tested on golf courses. Thus, combinations of entomopathogenic nematodes with the milky disease bacterium, *Paenibacillus popilliae* Dutky, chemical insecticides, and cultural methods have been tested. *Steinernema glaseri* Dongrae strain and *P. popilliae* caused 22 and 9% mortality of the 3rd instar of *E. orientalis*, respectively, whereas a combination of *S. glaseri* Dongrae strain and *P. popilliae* produced 90% mortality (Choo, unpublished data). A combination of one-half rate of *Heterorhabditis* sp. and one-half rate of chloropyrifos-methyl was synergistic, causing 91% mortality compared with 69% for the full rate of *Heterorhabditis* sp. or 22% for the full rate of chloropyrifos-methyl. In

addition, one-half rate of *S. longicaudum* Nonsan strain plus one-half rate of chloropyrifos-methyl caused 96.8% mortality compared with 45.9% for the full rate of *S. longicaudum* Nonsan strain and 28.7% for a full rate of chloropyrifos-methyl (Lee et al., 2002a).

Soil aeration improved nematode efficacy in the field. Thus, mortality of *E. orientalis* larvae could be increased from 16.2 ± 9.6% for *S. longicaudum* Gongju strain without aeration to 40.1 ± 4.8% for *S. longicaudum* Gongju strain plus aeration (Choo, unpublished data). However, aeration did not improve mortality with *Heterorhabditis* sp. as mortality was 88.8 ± 4.5% with aeration and 86.4 ± 10.9% without aeration. Application time also affected nematode efficacy. Pre-overwintering *E. orientalis* larvae were more susceptible than post-overwintering larvae (Lee et al., 2002a).

4.4.4. Commercialization

Commercial production has been initiated on a limited scale in Korea during the 1990s. The Sesil (http://www.sesilipm.co.kr), a company specializing in biological control agents, has started in vivo nematode production using the greater wax moth, *Galleria mellonella* (L.), larvae. The company produces 200 packs of *S. carpocapsae* Pocheon strain and 380 packs of an unidentified Korean isolate of *Heterorhabditis* sp. a day. The size of each pack is $30 \times 16.5 \times 3$ cm and includes 20×10^6 IJs formulated on sponge. The nematodes are sold for use against caterpillars on vegetables, fungus gnats on mushrooms and other insect pests of greenhouse plants.

4.5. Development of entomopathogenic nematode/bacterium research in Turkey

4.5.1. Background

Research on the entomopathogenic nematode/bacterium complex has only been recently initiated. The many isolates and species found in Turkey provide vast opportunities for conducting fundamental studies with this complex and for using them in biological control programs against a number of soil insect pests and insect pests in cryptic habitats (Hazir et al., 2003a).

4.5.2. Nematode diversity

Several surveys have been conducted with the isolation of several known species (Hazir et al., 2003b; Kepenekci, 2002; Kepenekci and Susurluk, 2003; Özer et al., 1995) and one new species (Hazir et al., 2003c). Özer et al. (1995) initially stated that they recovered *S. carpocapsae* from the coast of the Black Sea, but this species was later identified as *S. feltiae* (Hominick et al., 1996). However, Kepenekci (2002) isolated *S. carpocapsae* from a forest area of Anamur. Kepenekci and Susurluk (2003) found two *H. bacteriophora* isolates and *S. feltiae* from Ankara, and Hazir et al. (2003b) isolated *H. bacteriophora*, *S. feltiae*, *S. affine* (Bovien), and a new species *Steinernema anatoliense* Hazir et al. (2003c).

4.5.3. Ecological characterization

Susurluk et al. (2001) did an identification and ecological characterization of three nematode–bacterium complexes (one steinernematid and two heterorhabditid isolates) that were isolated on the University of Ankara campus. *Steinernema feltiae* was identified based on morphometric data and shape of the spicules, whereas the two heterorhabditid isolates (TUR-H1 and TUR-H2) were identified by molecular methods and by cross breeding tests with a known *H. bacteriophora* isolate. The RFLP analysis showed that these two isolates were members of the species *H. bacteriophora* and their bacterial symbionts shared >99% similarity in the 16S rDNA sequence with *P. luminescens* subsp. *laumondii*.

The infectivity at various soil moistures and heat tolerance of the three Turkish nematodes (TUR-H1 and TUR-H2 strains of *H. bacteriophora* and *S. feltiae*) were compared with each other (Susurluk et al., 2001). Using a nematode penetration assay, significantly higher infection rate was observed for all three nematode isolates at 10% soil moisture than at other soil moisture levels. From 7 to 10% soil moisture, more *S. feltiae* invaded the insect hosts than the two heterorhabditid isolates. Above 10% soil moisture, there was a reduction in all three nematode species invading the insect host. The heat tolerance study showed that *S. feltiae* was the most tolerant nematode at 32 °C, but no nematodes could survive 36 °C after 4- or 5-h exposure. In another study with TUR-H1 and TUR-H2, TUR-H2 had better host searching ability than TUR-H-1 at different temperatures (Susurluk et al., 2003).

Further research was conducted with the TUR-H2 strain of *H. bacteriophora* from Ankara and *S. feltiae* from the Black Sea region of Turkey (Oğuzoğlu Ünlu and Özer, 2003). At 25 °C, the IJs of *H. bacteriophora* and *S. feltiae* emerged from *G. mellonella* hosts 6 and 9 days post infection, respectively. Generally, heterorhabditid species emerge from cadavers 10 or more days post infection (Poinar, 1990; Wouts, 1984), and the rapid emergence of IJs of TUR-H2 strain presents an interesting biological difference with other isolates of *H. bacteriophora*.

The development of five geographic isolates of *S. feltiae* from Mediterranean (Sinop strain from Turkey; SN strain from France; and Monterey strain from California), subtropical (Rafaela strain from Argentina), and tropical (MG-14 strain from Hawaii) regions were compared at different temperature regimes (Hazir et al., 2001). The five isolates were exposed to 5, 8, 10, 15, 20, 25, and 28 °C in wax moth, *G. mellonella*, larvae and mortality and progeny production data were obtained. All isolates caused 100% mortality of wax moth larvae and developed and produced progeny between 8 and 25 °C, but at 28 °C, 100% mortality occurred with no progeny production. The highest IJ production was observed at 15 °C for all isolates with the Turkish Sinop isolate having the earliest emergence time from cadavers at 15 (10 days) and 20 °C (8 days).

4.5.4. Bacterial isolates

Two new subspecies of *Photorhabdus luminescens*, subsp. *kayaii* and *thraciaensis*, were described from Turkish isolates of *H. bacteriophora* (Hazir et al., 2004). Seven bacterial isolates were obtained from *H. bacteriophora*, and using riboprint analyses and metabolic properties, two isolates showed differences to represent subspecies status.

5. Central America

5.1. Background

In Latin America, research is done mainly in public universities and government research centers with funds from the National Councils of Science and Technology. However, in some Latin American countries, this council does not exist, and research is done independently or is non-existent. Even where research has been conducted on the nematode/bacterium complex, the results are not easily accessible because they are mainly in unpublished local congresses, dissertations, and technical reports, most of which are written in Spanish. Some institutions have web pages and some information can be retrieved from them, but often these have not been peer reviewed. Basically, research on entomopathogenic nematodes emphasizes the isolation of native strains, laboratory assays, and field trials for biological control of different pests and mass propagation of nematodes. In addition, Costa Rica has recently initiated a research program on entomopathogenic nematodes (Stock, pers. commun.).

5.2. Nematode diversity

Isolation of indigenous nematodes is promising because of the great biodiversity in these countries. For example, *S. carpocapsae* was discovered in Chihuahua (Poinar, 1990) and *H. indica* was isolated from a subtropical region in Mexico (La Sierra, Tabasco) (Cortez-Madrigal et al., 2003). *Heterorhabditis* sp. and *Steinernema* sp. were collected from semi-desert areas at an altitude higher than 2000 m in Zacatecas (Cortez-Madrigal et al., 2003). The latter two isolates survived temperatures between 30 and 32 °C and their thermotolerance increased after thermal adaptation (Lozano-Gutierrez et al., 2000a,b). *Heterorhabditis* sp. and *Steinernema* sp. were also isolated from 10.9% of baited soils in 64 locations sampled from maize, grain sorghum, forage sorghum, and Sudan grass fields in six Mexican states (Molina–Ochoa et al., 2003a), and in Jalisco, Mexico, a *Heterorhabditis* sp. was isolated from soil samples (Diaz-Mederos et al., 2002). In the Centro de Ciencias Medio Ambientales in El Salvador, an indigenous nematode strain was isolated from Alto Valle del Ebro and will be tested against *Agriotes* spp. (Coleoptera, Elateridae) in potato (Alia et al., 2002). In Costa Rica, two *Steinernema* isolates and *H. indica* have been recovered from soil samples (Uribe-Lorio et al., 2005). Ruiz-Vega et al. (2003) collected *S. feltiae* and an unidentified *Heterorhabditis* species from Oaxaca, Mexico. Recently, Nguyen et al. (2004) described a new species, *Heterorhabditis mexicana* Nguyen, Shapiro-Ilan, Stuart, McCoy, James and Adams from Tamaulipas. Shapiro-Ilan et al. (2005) have characterized some biological control traits of this newly described species.

5.3. Application against target pests

There is considerable interest in using these nematodes as biological control of important pests in Central America, and the information is summarized in Table 3. Briefly, *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae), which is an important pest of maize, sorghum, cotton, and soybean is susceptible to *H. megidis* (Molina–Ochoa et al., 2003b). White grubs, which are pests of maize, beans, sorghum, wheat, potato, tomato, chili, pineapple, mango, sugarcane, etc., may be managed by a combination of *H. bacteriophora* and an entomopathogenic fungus, *Metarhizium anisopliae* (Metch.) (Ruiz-Vega and Aquino-Bolaños, 2002). The lethal time to kill 50% (LT_{50}) of the white grubs was 2.3 weeks. Moreover, *S. feltiae* controlled white grubs in blue agave plantations (Chavez, pers. commun.).

In 1983, the hemipteran, *Cyrtomenus bergi* Froeschner, was detected in cassava fields in Panama with losses reaching as high as 70% of the crop. This pest occurs in Colombia, Panama, Brazil, Argentina, Venezuela, Costa Rica, Honduras, and Cuba, and has a wide host range including potato, peanut, asparagus, cotton, tomato, maize, coffee, banana, etc. In laboratory bioassays *S. carpocapsae* caused 59% of adult mortality 10 days after treatment, whereas a Colombian strain of *H. bacteriophora* killed 84% of the immature stages (Aguilar, 2003).

Coffee is an export product produced in several Central American countries, but its production has been reduced by attacks on the berry by the scolytid, *Hypothenemus hampei* Ferrari. Biological control research on this insect was initiated in the 1980s as a multinational program with parasitoids, entomopathogenic fungi and nematodes (*S. carpocapsae*, *Heterorhabditis* spp.), and a tylenchid nematode (Castillo and Marban, 1996; Castillo et al., 2002). As yet, there are no satisfactory biological control agents to suppress this important pest.

Although some of the nematode research has provided efficacious results against a number of insect pests in Central America, they are not available commercially. In the future, commercially produced nematodes may have use against insect pests in high value crops such as organic products, vegetables, flowers, and mushrooms.

5.4. Mass production

Entomopathogenic nematodes have been produced for research at the Centro de Nacional de Referencia para el Control Biologico (Tecoman, Colima, Mexico) and Colegio de Postgraduados (Texcoco, Mexico) in vivo and

Table 3
Target pests for entomopathogenic nematodes in some Central and South American countries

Order Family	Scientific and/or common name	Commodity	Country or region	Nematode sp.[a]	References
Lepidoptera					
Gelechiidae	*Tecia solanivora* Povolny	Potato	Colombia Venezuela	Sf Entomopathogenic nematodes	Alvarado et al. (1998), Saenz (2003), Fan et al. (2000)
Glyphipterigidae	*Sagalassa valida* Walker	Palm	Colombia	Sc	Ortiz-Sarmiento (1994)
Noctuidae	*Spodoptera frugiperda* (J.E. Smith)	Grains	Mexico	Hm	Molina–Ochoa et al. (2003b,c)
Noctuidae	*Helicoverpa zea* (Boddie)	Maize	Mexico	Sf	Molina–Ochoa et al. (2003a)
Noctuidae	*Heliothis virescens* (F.)	Cotton	Brazil	Sc, Sg, H sp. strain CCA	Ziani and Aguillera (unpublished data)
Noctuidae	*Anticarsia gemmatalis* (Hübner)	Soybean	Brazil	H sp. strain CCA	Negrisoli and Aguillera (unpublished data)
Coleoptera					
Scarabaeidae	White grubs	Maize	Mexico	Hb	Ruiz-Vega and Aquino-Bolaños (2002)
Scarabaeidae	White grubs	Agave	Mexico	Sf	Chavez (Pers. Comm.)
Curculionidae	*Cosmopolites sordidus* (Germar)	Banana	Brazil	Sc	Schmitt et al. (1992)
			Venezuela	Sc	Rosales and Suarez (1997)
Curculionidae	*Naupactus* sp.	Citrus	Brazil	H sp. strain CB-n5	Leite et al. (unpublished data)
Curculionidae	*Premnotrypes vorax* Hustache	Potato	Chile	S sp.	Garzon et al. (1996)
			Peru	H sp.	Alcazar (unpublished data)
Castniidae	*Castnia dedalus* Cramer	Palm	Suriname	Entomopathogenic nematodes	Segeren-Oever et al. (1984)
Scolytidae	*Hypothenemus hampei* (Ferrari)	Coffee	Coffee–growing areas	Sc, H sp.	Molina-Acevedo and Lopez-Núñez (2002, 2003)
Curculionidae	*Parapantomorus* sp.	Citrus	Brazil	Sa, Sg, S sp.	Garcia and Aguillera (unpublished data)
Cerambycidae	*Migdolus fryanus* Westwood	Sugarcane	Brazil	Sc	Arrigoni et al. (1986)
Hemiptera					
Cercopidae	*Mahanarva fimbriolata* (Stäl)	Sugarcane	Brazil	Sa, Sc, Sg, H sp. strain CCA	Leite et al. (2003)
Cydnidae	*Cyrtomenus bergi* Froeschner	Cassava and other crops	Panama	Sc, Hb	Aguilar (2003)

[a] Sa, *Steinernema arenarium*; Sc, *S. carpocapsae*; Sf, *S. feltiae*; Sg, *S. glaseri*; Ss, *S. scapterisci*; S sp., *Steinernema* sp.; Hb, *H. bacteriophora*; Hm, *H. megidis*; H sp., *Heterorhabditis* species.

in vitro on solid-state culture using chicken-offal. In addition, mass production of *S. carpocapsae* in liquid culture using an airlift bioreactor or a bubble column was developed at CINVESTAV-IPN (Mexico City). In the bioreactor studies, some basic engineering principles on the critical parameters to obtain high yields are being examined. Some findings indicate that the growth kinetics of *S. carpocapsae* in liquid culture can be modeled using a re-parameterized Gompertz model and the multiplication factor (ratio between initial and final concentration of nematodes), the time required from inoculation of the bioreactor until the first progeny produced, and the growth rate could be calculated (Chavarria-Hernandez and de la Torre, 2001). Interestingly, viscosity of the medium was similar to that of water during differentiation of the juvenile stages and reproduction of *S. carpocapsae*, but it increased towards the end of the process when the adults died and most of the populations consisted of IJs (Chavarria-Hernandez et al., 2003). The two most important engineering aspects for mass production of *S. carpocapsae* are (1) the oxygen transfer rate and (2) hydrodynamics to allow mating and avoiding mechanical damage of the second-stage juveniles (de la Torre, 2003). In a bubble column, the velocity of the bubbles was related to size; bigger bubbles traveled faster than the smaller ones. When a nematode became part of the bubble wave, it became isolated from the other nematodes and traveled 3 to 6 times faster than the other nematodes. Therefore, high velocities of both nematodes and bubbles appeared to inhibit mating and hence reproduction. A different distribution pattern of females and males favored mating and reproduction of *S. carpocapsae* in an airlift reactor (Neves et al., 2001). Accordingly, heterogeneous distribution of females and males depends on both bioreactor design and operation conditions. In a bubble column, the larger females accumulated at the bottom of the bioreactor when the bubbles from the air sparger were small and low air flows were used (Reyes, 2003). These studies are geared to develop large-scale in vitro commercial production of *S. carpocapsae*.

6. South America

6.1. Background

Presently, 7 of the 12 South American countries (as far as we are aware, none of the three territories conduct nematode research) are known to conduct research with entomopathogenic nematodes. Those countries with some level of entomopathogenic nematode activity are: Argentina, Brazil, Chile, Colombia, Peru, Suriname, and Venezuela. Most of the research comprises some morphological, taxonomic, biological, and ecological studies, soil/insect sampling, laboratory testing of local and exotic species against target insects, and preliminary studies on mass production. The published research on morphological, biological, and ecological studies by South American scientists is still limited. Some studies have focused on the life cycle of native isolates of *S. feltiae* (Saenz and Luque, 2000a) and *H. bacteriophora* (Doucet et al., 1996a). There is one study on the effects of temperature on morphometric characters of *H. bacteriophora* (Doucet et al., 1991). A study conducted in Argentina by Doucet et al. (1996b) about the effects of temperature and inoculum density on the efficacy of *H. bacteriophora* against *G. mellonella* showed that the infective juveniles from hermaphroditic females were more efficient to kill the host than those from amphimitic females. They also observed that the highest mortality occurred at 26 °C with a range from 12 to 36 °C. At high inoculum densities, higher mortality occurred at temperatures between 18 and 30 °C. In Colombia, Molina-Acevedo and Lopez-Núñez (2003) demonstrated that the addition of Tween or glycerin enhanced the ability of *S. feltiae* and *H. bacteriophora* to control the coffee borer, *Hypothenemus hampei*. The addition of Tween or glycerin served to protect the infective juveniles from desiccation and aided them in invading the coffee berry and allowing them to infect the host inside the fruit.

6.2. Nematode diversity

Most of South America remains unexplored for entomopathogenic nematodes. Yet, according to Poinar (1990), the first report on what may be a species belonging to the genus *Heterorhabditis* was initially described more than 65 years ago as *Rhabditis hambletoni* Pereira (Pereira, 1937). Subsequently, *S. glaseri*, a species described initially from North America, was recovered from the egg of *Migdolus fryanus* (Westwood) obtained from a field-collected female in the State of São Paulo, Brazil (Pizano et al., 1985). New species described from South America include *S. rarum* (Doucet, 1986) and *S. ritteri* Doucet and Doucet (1990), both of which were found in Argentina, and *S. scapterisci* which was isolated from the mole cricket *Scapteriscus vicinus* Scudder collected in Uruguay (Nguyen and Smart, 1990). The latest nematode to be described was *H. argentinensis* Stock (1993) isolated from *Graphognatus* sp. collected in Santa Fé, Argentina.

Although only few surveys for entomopathogenic nematodes have been carried out in South America, what has been discovered so far suggests a very rich and diverse nematode fauna. In Brazil, sampling from 1532 localities generated 18 isolates of *S. carpocapsae* and 13 isolates of *Heterorhabditis* (Fowler, 1988). *Steinernema feltiae* was also recovered from nematode-killed insects in the State of São Paulo (Fowler and Garcia, 1988). In Argentina, a comprehensive survey was conducted in the Pampean Region leading to the isolation of *S. feltiae*, *S. carpocapsae*, *S. scapterisci*, *H. bacteriophora*, and *H. argentinensis* Stock (Stock, 1992, 1995). In Venezuela, a survey was carried out in which six isolates of *Heterorhabditis* spp. were recovered including *H. indica* (Rosales and Suarez, 1998). In Peru, a cold-adapted heterorhabditid species was isolated from the potato weevil in the high Andes and shows biological control potential for this insect (Alcazar and Parsa, unpublished data). Another heterorhabditid nematode has been isolated from the coastal area. Molecular evidence suggests that both heterorhabditid isolates appear to be new species (Stock, pers. commun.). In other countries, *S. feltiae* was isolated in Colombia (Saenz, 1999), and an unidentified *Heterorhabditis* sp. was isolated in Venezuela (Fan et al., 2000) and Suriname (Segeren-Oever et al., 1984).

6.3. Application against target pests

Much of the research on entomopathogenic nematodes in South America examined the efficacy of non-native and native isolates of *Steinernema* spp. and/or *Heterorhabditis* spp. for the control of target insects, especially in the orders Lepidoptera and Coleoptera (Table 3). Thus, experiments on host range included insect species belonging to the orders Coleoptera, Lepidoptera, and Hymenoptera in Argentina (Doucet et al., 1999; Doucet and Giayetto, 1994; Stock, 1996). Similarly, experiments on host suitability have been conducted with the Brazilian isolates of *S. feltiae* showing that they were effective against the mole crickets, *Scapteriscus* spp. (Fowler, 1988; Fowler and Garcia, 1988). In laboratory tests, Vasconcelos et al. (2004) demonstrated that a concentration of 5000 infective juveniles of the Brazilian isolate of *S. glaseri* reduced egg deposition by engorged females of *Boophilus microplus* Canestrini (Acari: Ixodidae) by 90%. In comparison, 1500 infective juveniles of the Brazilian isolate of *H. bacteriophora* reduced egg deposition by 80%. Tick mortality increased linearly with increasing concentrations of *S. glaseri*, but this linear response was not observed with *H. bacteriophora*.

6.4. Mass production

Implementation of entomopathogenic nematodes for control of insect pests depends on the availability of inoculum of the desired nematode species/strains, especially for application in large areas. Some of the main crops with soil insect pests in South America include sugarcane, coffee, potato, and citrus, but these cropping systems will require

high amounts of inocula. Pest control in greenhouse represents a promising market segment for using entomopathogenic nematodes, especially because lower amounts of inoculum are required. Current production of entomopathogenic nematodes is primarily for laboratory and small-scale studies from nematodes produced in vivo using *G. mellonella* (Leite et al., 1990; Molina-Acevedo and Lopez-Núñez, 2001; Saenz and Luque, 2000b). The nematodes can also be reared on other hosts such as *Diatraea saccharalis* (F.) (Folegatti et al., 1988) and *Achroia grisella* F. (Saenz and Luque, 2000b). Success in mass producing these nematodes, especially those that are native isolates, economically constitutes an important step towards implementation in South America.

7. Africa

7.1. Nematode diversity

The African continent represents a fertile field for entomopathogenic nematode exploration because only a few countries have been surveyed. The few surveys for entomopathogenic nematodes have been documented with a number of new species and strains reported. New nematode species described from Africa include *S. karii* Waturu, Hunt and Reid (Waturu et al., 1997) from Kenya and *H. taysaerae* Shamseldean, Abou-El-Sooud, Abd-Elgawad and Saleh (Shamseldean et al., 1996a) from Egypt. New strains of *H. indica* and *H. bacteriophora* were reported from Kenya and Egypt (Burnell and Stock, 2000; Hominick, 2002; Stack et al., 2000). In addition, isolates of *Steinernema* and *Heterorhabditis* species have been recovered from South Africa (Spaull, 1991). These species and strains were recovered from a diverse range of environments. Isolates of *Heterorhabditis* species were recovered from grasslands in Uganda (Grewal et al., unpublished data), and entomopathogenic nematodes were isolated from more than 20 sites in a South African survey (Hattingly, unpublished data).

7.2. Application against target pests

Current research on the entomopathogenic nematode fauna of Africa is focused on their efficacy under laboratory and field conditions. In laboratory studies to determine the virulence of various strains of *S. carpocapsae* and *H. bacteriophora* against different developmental stages of African ticks (various species) in Namibia, Kaaya et al. (2000) observed that engorged female ticks were susceptible to the nematodes, whereas unfed females and immature stages were resistant to infection. Their data suggest that nematodes could be used for control of some African tick species.

Extensive work on nematodes has been done on survival, infectivity, and virulence in Egypt (e.g., Ghally, 1995; Shamseldean and Abd-Elgawad, 1995; Shamseldean et al., 1996b, 1998, 1999). These studies have shown promising results indicating that these nematodes could be developed for biological control and incorporated into integrated pest management programs in some cropping system in Egypt. In addition, studies on the compatibility of nematodes with other insecticidal agents have been reported. For example, Ayaad et al. (2001) found that injection of third larval instars of the flesh fly, *Parasarcophaga surcoufi* (Villeneuve), with *H. bacteriophora* HP88 and azadirachtin significantly suppressed hemolymph phenoloxidase activity of the larvae, even in the presence of laminarin, α-chrymotrypsin, and methanol activators. Furthermore, Salama and Abd-Elgawad (2002) investigated the use of Egyptian isolates of *Heterorhabditis* in combination with a virus for management of the red palm weevil, *Rhynchophorus ferrugineus*. They found that low viral concentrations enhanced nematode activity against *R. ferrugineus*. Studies have also been conducted on the effectiveness of different nematode formulations. Thus, Zaki et al. (1999) reported significant control of the cutworm, *Agrotis ipsilon*, using *S. carpocapsae* in a bait form compared with a suspension and irrigation water in field studies. The virulence of the bacterial symbiont, *X. nematophila* from *S. carpocapsae*, was greater than that of *P. luminescens* from *H. bacteriophora* against the diamondback moth, *Plutella xylostella*, pupae in Egypt (Abdel-Razek, 2003). Elsewhere, Akalach and Wright (1995) reported a significant reduction in larvae of *Conorrhynchus mendicus* (Gyll.), a weevil pest of sugar beets, in laboratory and field studies in Morocco using *S. carpocapsae* and *S. feltiae*.

While the above studies report successes, limited field successes in insect pest control have been reported against the lepidopteran sugarcane stalk borer, *Eldana saccharina* Walker, in South Africa (Spaull, 1991, 1992) and *R. ferrugineus* in Egypt (Salama and Abd-Elgawad, 2001). The apparent failure in these two cases was attributed to the tunneling behavior of the larvae of these pests and sap in the infested sites in the stems and trunks. (However, *R. ferrugineus* was controlled by *S. carpocapsae* in Japan; see Section 4.3.2.) Although commercial applications have not yet been reported from Africa, plans are underway to develop an entomopathogenic nematode-based bioinsecticide using endemic strains for pest control in South Africa.

8. Conclusions

Entomopathogenic nematode research is being conducted in many different countries throughout the world. We have reported on the research from only a few countries and/or regions and have demonstrated the breadth of activities. Clearly, there is a great deal of interest in many countries in conducting research with these nematodes. As more scientists become trained in working with these entomopathogenic nematodes, the amount of published information will increase concomitantly.

Much of the recent research activities on the nematode–bacterium complex from Europe, North America, and Israel are published in other reviews. Therefore, we

concentrated on the commercial production in Europe and North America and then focused on research from a number of less represented research activities in different parts of the world.

In some countries such as China and India, there is active research on the nematodes and the symbiotic bacteria. In Korea, a great amount of basic and applied research has been conducted, and a commercial product is being produced in vivo on a limited scale. In Turkey, a number of new isolates have been found and are being tested against soil pests. In Mexico, emphasis is being placed on testing the nematodes against pest species and on in vitro production, but no commercial product is yet available; in Costa Rica, several new entomopathogenic nematode isolates have been found. The situation in most countries in South America shows an emerging interest in entomopathogenic nematodes with a number of new isolates being found. Some of these isolates are being evaluated against various soil pests, but with the exception of research in Argentina, much of the information is not in refereed journals. Similarly, in Africa, active research occurs in Egypt and there is some interest in South Africa, Uganda and Kenya, but most other countries have little entomopathogenic nematode research activity.

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