16 Status of Entomopathogenic Nematodes in Integrated Pest Management Strategies in Argentina

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16.1 Introduction

Entomopathogenic nematodes (EPNs) are one of the highly influential biocontrol agents regulating the population dynamics of insect pests through association with their hosts in relationships ranging from fortuitous to parasitic. Knowledge of nematode community is useful to interpret the host–parasite relationships, and in many cases to illustrate co-evolution phenomena and to determine those potential biocontrol agents against major pests of health and agricultural interest.

The Republic of Argentina is located in the Southern Cone of South America. Due to its large area, the latitude is characterized by distinct forms of relief: the mountains to the west and the plateaus, plains and depressions eastwards. This country presents a great diversity of climates and soils where the livestock and agriculture constitute the main economic activities. The quantity and quality of agricultural performance place Argentina as an important food producer that corresponds mostly with the sown area of cereals. The most widespread are wheat and maize, besides oats, barley, rice and sorghum. Also, oil and fibre plants, sugarcane, grapes, mate, tea, snuff, fruit and vegetables are grown.

Within this context, several studies have been performed in Argentina with the aim of isolating entomonematodes as biocontrol agents of pests of public health and agricultural importance. A great diversity of species has been reported as the result of research carried out during the past 35 years. The studies focused mainly on the description of numerous species, determination of biological cycles, ecology of pathogenic species and dynamics in natural conditions, mainly for Mermithidae, Thelastomatidae, Rhabditidae and EPNs Heterorhabditidae and Steinernematidae (Doucet, 1986; Doucet and Bertolotti, 1996; Lax *et al*., 2011; Achinelly and Micieli, 2013; Del Valle *et al*., 2013; Camino *et al*., 2014; Belaich *et al*., 2015; Di Battista *et al*., 2015). However, the research of this group of parasites was not commensurate to its potential. The lack of legislation regarding the regulation and registration requirements of different groups of bioproducts, the development of criteria or policy recommendations for studies to field or releases of new bioproducts, along with the weakening of the economy, have restricted the growth of this topic in Argentina. For these reasons, the studies have been limited mainly to laboratory stage or small field scale, leaving the development of entomonematodes as biocontrol agents in this country still incipient.

This chapter presents the results of research undertaken in Argentina and the current status of the potential use of entomonematodes as biologic control agents. Our main emphasis is inclined towards the two EPNs, *Steinernema* and *Heterorhabditis*, and their status in integrated pest management (IPM) in Argentina. The information presented joins the most relevant bibliographic information from the

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personal experience of the authors and other specialists in the country.

16.2 Integrated Pest Management in Argentina

IPM began to take hold in the 1990s as a decision system based on the selection and use of tactical control combined singly or harmoniously into a strategy related to the analysis of the cost/benefit ratio. The interests of farmers and the impacts on human health, society and the environment have been taken into account. Now, IPM is more related to the combination of technical expertise and economic analysis.

Currently, various government agencies such as INTA (National Agricultural Technology Institute) encourage the creation of consortia of integrated pest management (ConMIP) through projects in extensive crops.

16.3 Application of Biological Control Agents in Argentina

The growing interest in environmental quality has enhanced in recent years the search for new tools to control insects of agricultural importance that are safe, healthy and compatible with the environment. In Argentina, the study of parasites and pathogens began in the early 1980s, currently with a growing demand for bioinsecticides based on entomopathogens to control agricultural pests and disease vectors. The application of these agents was not commensurate with their potential, with successful results using the bacterium *Bacillus thuringiensis* to control agricultural and forestry pests, the microsporidium *Paranosema locustae* against locusts and the granulosis virus (*Betabaculovirus*; Baculoviridae) to control lepidopteran pest of fruit and nut trees (Sciocco-Cap, 2013). However, there are no registered products based on EPNs, and only a few nematodes have been evaluated at the small field scale.

16.4 An Outline of Studies on Entomonematodes (Parasites, Parasitoids and Pathogenic) in Argentinian Research Centres

A great diversity of nematode species with different associations with insects (parasites, parasitoids and pathogens) have been described in Argentina through research carried out during the past 40 years. A few groups can be mentioned: the first initiated by Dr Magdalena Agüera, at Centro de Zoología Aplicada, National University of Córdoba, continuing with the research of Drs Marcelo Doucet and Paola Lax and their working group in the province of Cordoba; a second group in Buenos Aires province, which began with Drs Stock and Camino following this subject uninterruptedly since 1980 until the present, at CEPAVE, CONICET, National University of La Plata; and a third in Santa Fe province with Dr Eleodoro Del Valle, who trained with researchers such as Dr Claudia Dolinski from Brazil, a specialist in EPNs.

Among the prominent contributions are the description of numerous species, the determination of biological cycles, the ecology of the pathogenic species and the dynamics under natural conditions – mainly for the Mermithidae, Thelastomatidae and Rhabditidae, as well as the EPNs Heterorhabditidae and Steinernematidae.

16.4.1 Entomopathogenic nematodes in insects impacting public health

Although there are no reports of EPNs isolated from insects impacting public health from Argentina, several studies have tested their efficacy. Infectivity was evaluated against mosquitoes, flies and lice. Two isolates of EPNs from Cordoba province were tested under laboratory conditions. A mortality of 84% was produced by *Heterorhabditis bacteriophora* in *Aedes aegypti* mosquito larvae at a dose of 750:1 infective juveniles (IJs)/larvae (Peschiutta *et al*., 2014). In the same way, *Culex apicinus* mosquito larvae were susceptible to *Steinernema rarum*, with higher mortality (75%) at a dose of 400:1 IJs/larvae (Cagnolo and Almirón, 2010). Nematodes of the two entomopathogenic families, Steinernematidae and Heterorhabditidae, produced mortality in lice. Strains of *H. bacteriophora* and *S. rarum* were able to infect specimens of *Pediculus humanus capitis*. Other mosquito species were susceptible to EPNs, such as *Culex quinquefasciatus* and *Ae. aegypti* larvae (*S. rarum*), and *Culex pipiens* (*Steinernema feltiae*). Pathogenicity by *S. feltiae* and *S. rarum* to *Musca domestica* larvae has also been reported (Doucet *et al*., 2008).

Parasitoids

Parasitoids are organisms that spend a significant portion of their life history in a single host in a relationship that is in essence parasitic; but they can also sterilize, kill and sometimes consume the host.

Mermithidae, a group of nematode parasites, principally of insects, and almost always lethal to the host, are considered parasitoids, where the host undergoes behavioural changes and is usually killed on mermithid emergence (Wise de Valdez, 2007). Several characteristics of these mermithid nematodes, such as environmental safety, host specificity, mass rearing *in vivo*, lethality, facile establishment and ready recycling, make them attractive biological control agents for mosquitoes (Platzer, 2007).

One of the first records of entomonematodes in Argentina was made by Berg (1898), who reported the presence of nematodes in locusts from Buenos Aires and Córdoba, the descriptions of which may well correspond to the family Mermithidae.

MERMITHID NEMATODES ISOLATED FROM MOSQUITOES (DIPTERA: CULICIDAE). Mermithid parasites of mosquitoes in Argentina were isolated from two species, constituting the only reports for the Neotropical Region: Poinar and Camino (1986) for *Strelkovimermis spiculatus*, with the floodwater mosquito, *Aedes albifasciatus* (Macquart), and Camino (1989) for *Hydromermis* sp. with *Psorophora ferox* larvae.

Several studies tending to use *S. spiculatus* as bioregulator of mosquito populations have been carried out in Argentina, and these are summarized below.

Strelkovimermis spiculatus *Poinar and Camino, 1986.* *S. spiculatus* was found to infect the host mosquito *Ae. albifasciatus* in rain-flooded ponds (Micieli *et al.*, 2012b) and *Cx. pipiens* in house-drainage ditches (Garcia and Camino, 1990; Muttis *et al.*, 2013) in the Buenos Aires province, Argentina. These insects are implicated in the transmission of arboviral and parasitic diseases worldwide, including extremely serious illnesses in humans, a reality that stimulates the study and development of strategies oriented around the control of these two mosquitoes. Because of the insect lethality of this nematode and the capacity to tolerate environments of elevated salinity and organic pollution, *S. spiculatus* has been identified as a possible biological control agent for culicine mosquitoes.

Studies have been performed on the life cycle and production of *S. spiculatus* (Camino and Reboredo, 1994, 1996), on the effect of certain biotic and abiotic conditions on the parasitism of *S. spiculatus* (Camino and García, 1991; Achinelly and García, 2003; Achinelly *et al.*, 2003; Micieli *et al.*, 2012a) and on the host–parasite interaction (Micieli and García, 1999; Campos and Sy, 2003; Micieli *et al.*, 2012b).

The life cycle of *S. spiculatus*, at an approximate duration of 35 days, is similar to that of other aquatic mermithids (Camino and Reboredo, 1994). Pre-parasitic but infective second-stage juveniles (J_2s) hatch from eggs and actively seek to penetrate the mosquito larvae. The third-stage juvenile (J_3) then develops in the mosquito larvae for 6–8 days, at which time the post-parasitic fourth-stage juvenile (J_4) emerges, killing the host. Finally, the females that become adults lay eggs in the aquatic substrate to complete the reproductive cycle (Fig. 16.1).

S. spiculatus was found to be parasitic on 16 mosquito species in Argentina under natural and laboratory conditions (Table 16.1). Epizootic levels were registered on only natural populations of mosquito, *Ae. albifasciatus*, with levels of infection reaching between 80 and 100% (Micieli and García, 1999; Micieli *et al.*, 2012b). Studies on this mermithid under laboratory conditions have indicated a wide range of susceptible mosquito species. This mermithid was observed to have completed development inside mosquito larvae and then emerge, resulting in lethality to all the mosquito larvae thus infected. The main difference in the host susceptibility was the degree of infection with respect to the instar phase, being notably more intense in the younger larvae (i.e. the first and second instars) at an overall mean infectivity of 80%, and ranging from 13 to 100%; much lower in the older larvae (i.e. the third and fourth instars) at a mean value of 38%, and ranging from 0 to 100% (Achinelly *et al.*, 2004).

S. spiculatus infections were not recorded with the majority of the non-target aquatic organisms exposed to the pre-parasites, with the sole exception of dipterans of the family Chironomidae: in which species, however, nematode penetration was detected in only 7% of the larvae exposed to the pre-parasites (Achinelly *et al.*, 2004). All nematodes that penetrated Chironomidae larvae died at 24–48 h post-infection through host resistance in the form of melanization. This nematode was also introduced in natural populations of culicids: *Ae. aegypti*, *Cx. apicinus* and *Cx. pipiens* larvae (Achinelly and Micieli, 2009). The levels of parasitism fluctuated greatly between instar stages, sites and parasite doses. The overall parasitism observed for *Ae.*

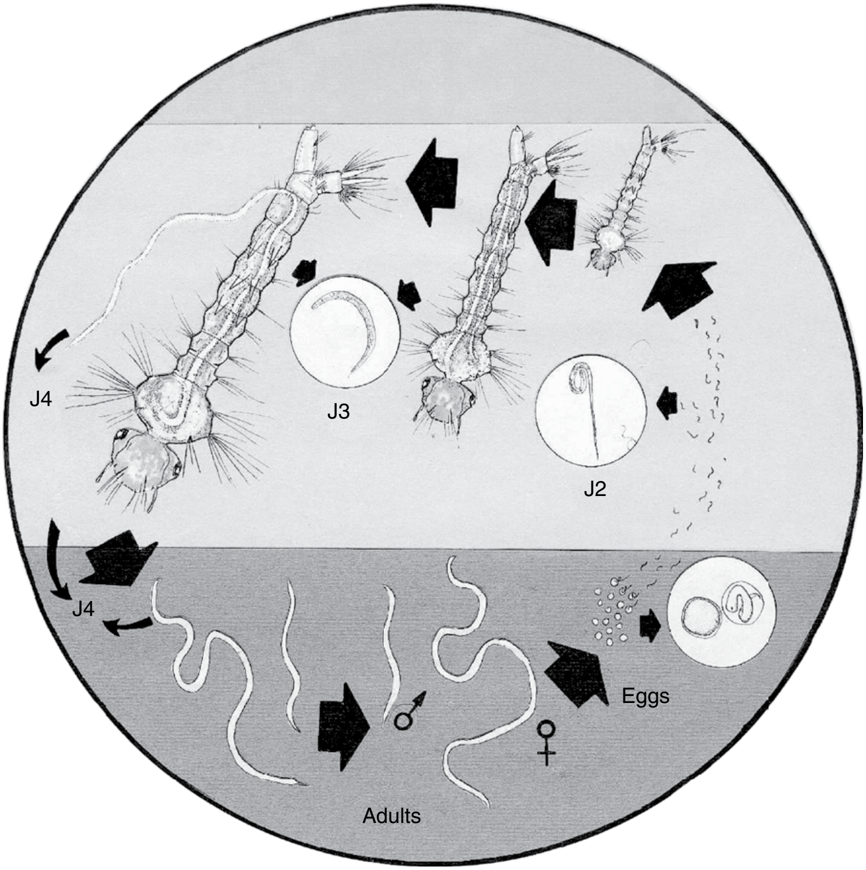

Fig. 16.1. Biological cycle of the EPN *Strelkovimermis spiculatus*: J_1, pre-parasitic juvenile inside an egg; J_2, pre-parasitic but infectious stage; J_3, parasitic stage; J4: free-living post-parasitic state.

aegypti, *Cx. pipiens* and *Cx. apicinus* was between 11–100%, 14–70% and 20–80% at dose ratios of 10:1, 50:1 and 100:1 J_2s/larvae, respectively; but the per cent infectivity among the three mosquito species was not significantly different at any given dose. The J_2s of *S. spiculatus* were observed in the field for up to approximately 10 days; which survival period agrees with the data of Micieli and García (1999), who had observed *S. spiculatus* J_2s alive for more than 7 days. Thus, mosquitoes with overlapping generations could be infected at different times with just one application of the nematodes in the breeding sites. This study constituted the first field evaluation of *S. spiculatus* with natural mosquito populations (Achinelly and Micieli, 2009).

From the above, *S. spiculatus* appears to be a promising potential biological control agent because of the mermithid's epizootic capabilities, its wide range of mosquito hosts and its innocuousness to non-target aquatic organisms. Nevertheless, certain parameters, such as the effect of this nematode's presence on water quality, should be considered before inoculating this entomoparasite in the field.

MERMITHID NEMATODES ISOLATED FROM SIMULIDS AND CHIRONOMIDS. Research in Argentina has included the characterization and description of new genera and species of mermithids that parasitize black flies in different wetlands in the following nine provinces of the country: Buenos Aires, Córdoba, Entre Ríos, Misiones, Tucumán, Neuquén, Río Negro, Jujuy and Mendoza. The resulting records from different locations in these provinces comprised two species of *Ditremamermis* Camino and Poinar, 1988; eight of *Mesomermis* Daday, 1911; four of *Gastromermis* Micoletzky, 1923; two of

Table 16.1. Spectrum of culicid hosts for the nematode *Strelkovimermis spiculatus* from Argentina. (Adapted from Achinelly and Micieli, 2013).

Mosquito larvae	Natural infections	Experimental infections	Laboratory infections
Aedes aegypti		x	x
A. albifasciatus	x		x
Anopheles albitarsis			x
Culex apicinus		x	x
C. castroi			x
C. chidesteri	x		x
C. dolosus	x		x
C. maxi	x		x
C. mollis	x		
C. pipiens	x	x	x
C. renatoi			x
Isostomya paranensis			x
Ochlerotatus crinifer	x		x
Psorophora ferox			x
P. ciliata	x		
P. cyanescens	x		

Octomyomermis Johnson, 1963; two of *Isomermis* Coman, 1953; two of *Limnomermis* Daday, 1911; one of *Hydromermis* Corti, 1902; and one of *Bathymermis* Daday, 1911.

Mermithids were also the only nematode family isolated from chironomid larval haemocoels. Three species of *Octomyomermis* genus and two of *Isomermis* sp. were recorded in the Buenos Aires province (Camino *et al.*, 2013b). They were always lethal for their hosts.

Parasites

Nematode parasites would not be as efficient as other families mentioned for use as agents of biological control of insects. However, it is worth mentioning an extensive study that was carried out in Argentina as a result of surveying and prospecting for entomonematodes.

NEMATODE PARASITES OF COCKROACHES. A survey of entomonematodes in cockroaches was conducted in order to find a nematode able to control this pest in Argentina (Table 16.2). Nematodes were isolated from *Periplaneta americana*, *Periplaneta brunnea*, *Periplaneta fuliginosa*, *Blatella vaga* and *Blatella germanica* (Lax *et al.*, 2008; Blanco *et al.*, 2012; Camino and González, 2012; Camino and de Villalobos, 2013; Camino *et al.*, 2013a; Gutierrez, 2015). All parasites isolated belong to the Oxyurida order, presenting in a location in the digestive tract without causing major changes in their hosts. *P. americana* presented the greatest diversity of parasite species. Parasitism in this host reached 70% in some cases.

16.4.2 Entomonematodes in insects of agricultural impact

Parasites and parasitoids

NEMATODES ISOLATED FROM GRASSHOPPERS, CRICKETS AND MOLE CRICKETS (ORTHOPTERA). The pampean region in Argentina is a major farming area where agricultural products are obtained for both domestic consumption and export, and where different species of grasshoppers exhibit outbreaks of significant magnitude, generating substantial economic losses. This environment was found to present a wide diversity of mermithid nematodes that might exert a form of regulation on the insect populations (Table 16.3). Entomonematode parasites of crickets and mole crickets were also registered in the Córdoba, Entre Ríos and Chaco provinces of Argentina (Camino and Stock, 1994; Camino *et al.*, 2013a), but only the Mermithidae family was lethal.

NEMATODES ISOLATED FROM SOIL PEST WHITE GRUBS (SCARABAEIDAE). A survey of nematode parasites and pathogens of white grubs in wheat fields of the pampas, Argentina, identified 14 species of nematode belonging to 5 families (Mermithidae, Thelastomatidae, Travassonematidae, Diplogasteridae and Rhabditidae (Table 16.4). The information on the nematode community was useful for interpreting host–parasite relationships. This study provides for the first time a list of parasitic nematodes of white worms of the pampas region of the country (Camino *et al.*, 2014).

Table 16.2. Nematodes (Oxyurida) parasitizing cockroaches in Argentina.

Host	Parasite
Periplaneta americana	*Thelastoma domesticus*
	Thelastoma sp.
	Hammerschmidtiella laplatae
	Hammerschmidtiella sp.
	Blattophila sp.
	Leidynema sp.
P. brunnea	*H. ettalaensis*
P. fuliginosa	*Hammerschmidtiella* sp.
	Leidynema appendiculata
Blatella vaga	*Protellus blatta*
B. germanica	*Blatticola* sp.

Entomopathogenic nematodes

EPNs (Rhabditida: Steinernematidae, Heterorhabditidae) are obligate parasites of a wide range of soil insects. Specimens of both families naturally regulate populations of various insects, behave as obligate parasites and kill their hosts.

They are considered pathogens because of a symbiotic relationship with bacteria causing death to insects. EPNs have been found all over the world in a range of diverse habitats. The cycle of life is characterized by a free-stage infective juvenile (IJ), which locates and penetrates the host body in a passive way, *Steinernema* spp., or in an active way, *Heterorhabditis* spp., through a tooth. When reaching the haemocoel, IJs release their symbiotic bacteria,

Table 16.3. Biodiversity of insect parasitic nematodes in soil pest (Orthoptera) in Buenos Aires, Argentina.

Host		Parasite	
Acrididae	*Laplatacris dispar*	Mermithidae	*Agamermis decaudata*
	L. dispar	Mermithidae	*Amphimermis bonaerensis*
	Dichroplus elongatus	Mermithidae	*A. dichroplusi*
	Metaleptera brevicornis	Mermithidae	*A. ronderosi*
	D. elongatus	Mermithidae	*Hexamermis cochlearius*
	Staurorhectus longicornis	Mermithidae	*H. ovistriata*
	L. dispar	Mermithidae	*Longimermis acridophila*
Gryllidae	*Gryllodes laplatae*	Mermithidae	*Amphimermis* sp.
		Mermithidae	*H. macrostomata*
		Thelastomatidae	*Blatticola cristovata*
		Thelastomatidae	*Cameronia laplatae*
		Cephalobiidae	*Cephalobium bidentata*
		Cephalobiidae	*C. dispar*
		Cephalobiidae	*C. laplata*
		Cephalobiidae	*C. magdalensis*
		Cephalobiidae	*C. tridentata*
		Cephalobiidae	*C. odontolateralis*
		Thelastomatidae	*Neyraiella distinctus*
		Diplogasteridae	*Mikoletzkya* sp.
		Rhabditidae	*Alloionema* sp.
		Rhabditidae	*Pelodera* sp.
		Rhabditidae	*Cruznema lincolnensis*
Gryllotalpidae	*Neocurtilla claraziana*	Mermithidae	*Agamermis decaudata*
		Thelastomatidae	*Cephalobellus cyclocephalae*
		Thelastomatidae	*Euryconema brevicauda*
		Thelastomatidae	*Fontanema gracilis*
		Thelastomatidae	*Gryllophila cephalobulata*
		Thelastomatidae	*Schwenkiella tetradentatum*
		Travassosinematidae	*Binema bonaerensis*

Table 16.4. Entomonematode parasitises of white grubs (Scarabaeidae), an important pest in crops of Buenos Aires, Argentina.

	Scarabaeidae			
	Cyclocephala signaticolis		*Diloboderus abderus*	
Parasites	Prevalence %	Intensity (number of parasites per infected insect)	Prevalence (%)	Intensity (number of parasites per infected insect)
Mermithidae				
Amphidomermis sp.	–	–	12	5.6
Hexamermis gracilis	–	–	10	5.5
Thelastomatidae				
Cephalobellus cyclocephalae	12	1.2	–	–
Cranifera robustum	10			
Severianoia brevicauda	–	–	60	1
Thelastoma modestus	–	–	65	30
T. rara	–	–	80	2500
Travassosinematidae				
Mirzaiella americana	–	–	32	1
Diplogasteridae				
Diplogaster octodontus	–	–	26	2.46
Noteodiplogaster papillosa	–	–	36	2.7
Patanodontus acaudatum	–	–	26	2.5
Rhabditidae				
Cruznema campestris	–	–	26	30
Parasitorhabditis platidontus	–	–	30	22
Rhabditis bonaerensis	–	–	26	17

which multiply rapidly, leading to septicaemia that kills the host within 24–48 h post-infection. The IJs are associated with symbiotic bacteria, *Xenorhabdus* spp. in Steinernematidae and *Photorhabdus* spp. in Heterorhabditidae, which multiply and generate metabolites that kill the insect and serve as a source of food for nematodes. Inside the host, IJs feed on bacteria, until reaching the adult stage. In case of Heterorhabditids, first generation is hermaphroditic but the following generations are amphimictics. But in Steinernematids all generations are always amphimictic, and they do not have hermaphrodictic generations.

In every generation, new JIs are produced, and the reproduction of the nematodes continues as long as the body of the insect continues to provide the necessary nutrients to the parasite. After leaving the body of the insect, they will move on to the ground to contact a new host. For both nematode genres, the bodies undergo a particular process, preventing invasion by other microorganisms and the consequent putrefaction by the antibiotics production for symbiotic bacteria. These nematodes provide an environmentally safe and economically reasonable alternative against a variety of important insect pests, and some of them are used commercially for biocontrol.

In Argentina seven species of the genus *Steinernema* and two of *Heterorhabditis* have been cited and studied, with more than 30 different isolations (Fig. 16.2). EPNs are widely distributed in this country, with records for the provinces of Córdoba, Neuquén, Río Negro, Santa Fe, La Pampa and Buenos Aires (Doucet, 1986; Doucet and Bertolotti, 1996; Doucet and Laumond, 1996; Doucet *et al.*, 1992, 2008; Stock, 1993a,b; Giayetto and Cichón, 2006). Researches have been oriented mainly on the biology and ecology (Doucet and Bertolotti, 1996; Lax *et al.*, 2011; Eliceche *et al.*, 2012; Achinelly *et al.*, 2013), the intraspecific variability (Doucet *et al.*, 1992, 1996) and the description of new species (Doucet, 1986; de Doucet and Doucet, 1990).

Field and laboratory assays demonstrated that insects from 11 orders and 42 families were susceptible to these nematodes. Studies on the parameters involved in EPN parasitism determined that the efficiency depended on the search strategies of the

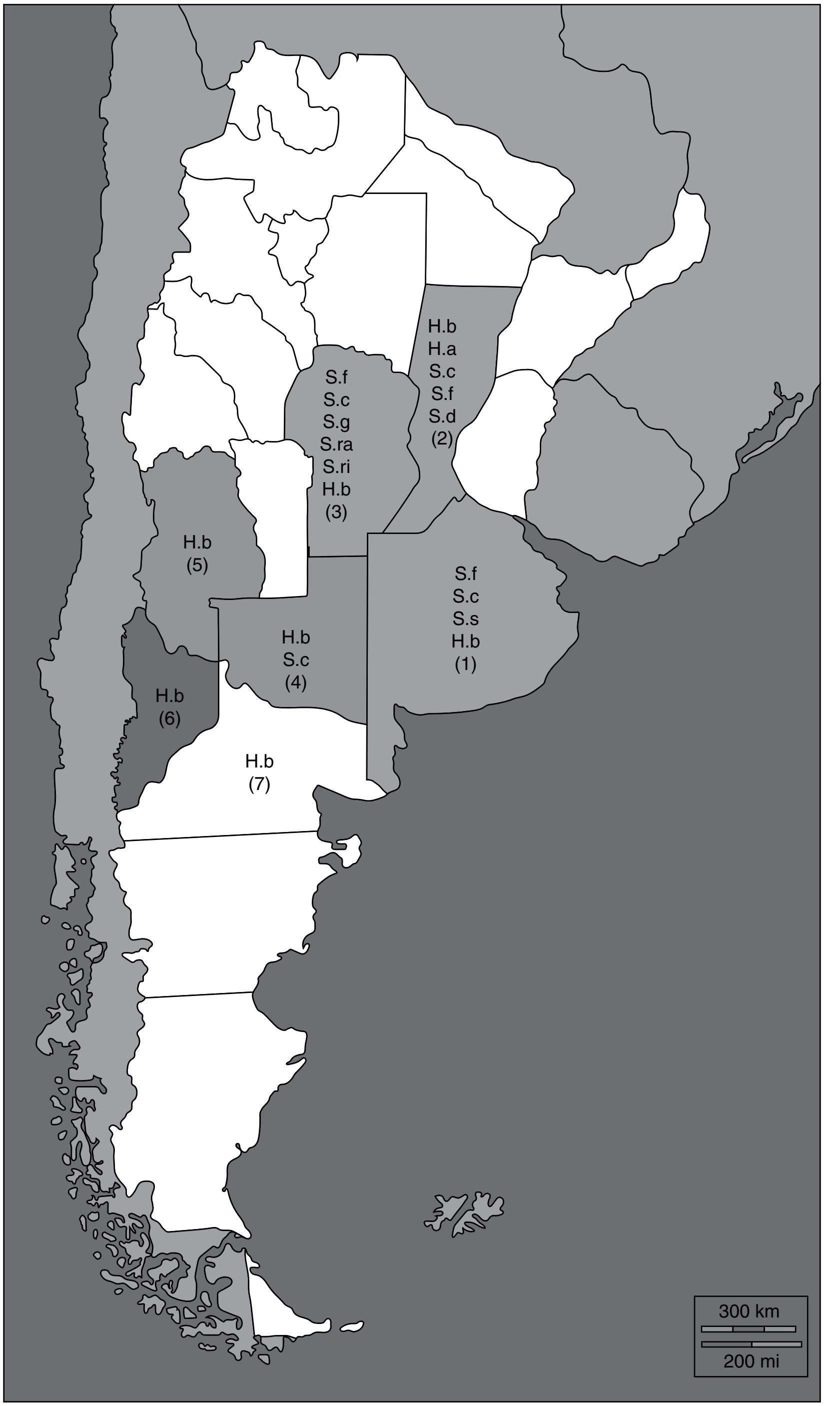

Fig. 16.2. Entomopathogenic nematodes from provinces of Argentina. 1 = Buenos Aires; 2 = Santa Fe; 3 = Córdoba; 4 = La Pampa; 5 = Mendoza; 6 = Neuquen; 7 = Rio Negro. H.a = *Heterorhabditis argentinensis*; H.b = *Heterorhabditis bacteriophora*; S.c = *Steinernema carpocapsae*; S.d = *Steinernema diaprepesi*; S.f = *Steinernema feltiae*; S.g = *Steinernema glaseri*; S.ra = *Steinernema rarum*; S.ri = *Steinernema ritteri*; S.s = *Steinernema scapterisci*.

IJs, along with their ability to remain in the environment, withstand stress conditions and become established in specific ambiences by adapting to the life cycle of their hosts (Doucet *et al*., 1992; Doucet and Bertolloti, 1996).

STEINERNEMATIDAE. The *Steinernema* genus includes adults with an excretory pore usually opening posterior to the basal bulb, males with copulatory papillae typically comprising a single midventral, precloacal papilla plus 10–14 paired papillae, bursa absent, females can present epitygmata and IJs with symbiotic bacteria usually visible in the intestinal pouch just posterior to the basal bulb. Both males and females are necessary for reproduction (amphimictic generations). Insects parasitized by *Steinernema* spp. usually show brown and eventually darker black colours, although they have also been observed with pink, yellow and green grey colours.

Seven species of the genus *Steinernema* have been isolated from Argentina: *Steinernema carpocapsae* (Weiser, 1955) with nine isolates; *S. feltiae* (Filipjev, 1934) Wouts, (seven isolates); *Steinernema diaprepesi* Nguyen and Duncan (one isolate); *Steinernema glaseri* (Steiner, 1929) (one isolate); *Steinernema ritteri* (de Doucet and Doucet, 1990) (two isolates); *S. rarum* (Doucet, 1986) Mamiya, 1988 (nine isolates) and *Steinernema scapterisci* (Nguyen and Smart, 1990) (two isolates); with records from Córdoba, Santa Fe and Buenos Aires provinces (Doucet, 1986; de Doucet and Doucet, 1990; Stock, 1992, 1993a, 1995; Lax *et al*., 2011). Among these citations, *S. rarum* and *S. ritteri* were described for the first time in Argentina (Doucet, 1986; de Doucet and Doucet, 1990). These *Steinernerma* species were all found in cultivated soils, with the sole exception of *S. glaseri*, with that specimen having been isolated from mountain forest sod (Doucet *et al*., 2008).

Pathogenicity and host range. Several research workers have evaluated steinernematid nematodes against a wide range of insect pests, presented below (Table 16.5).

Recent studies have demonstrated the efficacy against lepidopterans of economic significance. An *S. rarum* isolate (OLI strain) produced mortalities of 96% and 98% in *Anticarsia gemmatalis* and *Crocidosema aporema* larvae, respectively, at a dose of 500 IJs/insect. The life cycle was shorter in *C. aporema*, whereas production was longer (4–7 days) in *A. gemmatalis* (Gianfelici *et al*., 2014). Adults of *A. gemmatalis* were also susceptible to this nematode species with a high mortality of 80% at a dose of 500 IJs/adult (Cagnolo *et al*., 2010).

S. diaprepesi was also evaluated against the lepidopterans *Spodoptera frugiperda* and *Helicoverpa gelotopoeon*. Infections were performed individually in Petri dishes (35 mm diameter) by applying IJs immersed in 0.5 ml water at 50 and 100 IJs/dish, each treatment contained 15 larvae. Mortality of 100% and 93% was obtained with the highest dose in *S. frugiperda* and *H. gelotopoeon*, respectively (Caccia *et al*., 2014). In addition, studies on the pathogenicity of *S. carpocapsae* and *S. rarum* indicated the potential of these species as biocontrol agents for insects belonging to the orders Hemiptera, Diptera and Coleoptera (Bertolotti *et al*., 2007; Doucet *et al*., 2008; Lax *et al*., 2011).

HETERORHABDITIDAE. The genus *Heterorhabditis* includes males usually with nine pairs of genital papillae, bursa present, IJs with dorsal tooth in the cephalic region, symbiotic bacteria retained in intestine, first-generation adults hermaphroditic, second-generation adults amphimictic.

Insects killed by *Heterorhabditis* spp. usually have colorations ranging from reddish-brown, orange and purple, and may have luminescence in the dark, depending on the bacteria of the genus *Photorhabdus*.

Two nematode species belonging to the genus *Heterorhabditis* have been registered in Argentina (see Fig. 16.2): one population of *Heterorhabditis argentinensis* Stock, 1993, in Rafaela, Santa Fe province, plus several populations of *H. bacteriophora* Poinar, 1976 – along with additional records of that species for the provinces of Córdoba (OLI, RIV strains), valleys of the Patagonian region, Neuquén and Río Negro provinces (RN, Nq, Biodiv., INTA A-V; INTA 11-12; ROCA strains), Mendoza (Rama Caída), Santa Fe (Juan and Mo strains), La Pampa and Buenos Aires (VELI) (Stock, 1993b; Doucet and Bertollotti, 1996; Giayetto *et al*., 1998; Doucet *et al*., 2008; Del Valle *et al*., 2013).

The genetic proximity of *H. bacteriophora* to *H. argentinensis* has been discussed by several authors. A careful analysis of the rDNA internal transcribed spacer ITS-1 characters revealed that these closely related sister taxa would be conspecific, differing by a single transition and deletion (Adams *et al*., 1998).

Pathogenicity and host range. A wide range of susceptible insects to heterorhabditid nematodes have been registered under laboratory and field conditions, including eight orders and 27 families (Table 16.6). Mortality was achieved in all organisms tested, completing the life cycle with rare exceptions (Thysanoptera)

Table 16.5. Spectrum of experimental and naturally infected susceptible hosts to entomopathogenic nematodes (Steinernematidae). (Adapted from Doucet *et al.*, 2008).[a]

Host range			Nematode	Isolate	References
Anoplura	Pediculidae	*Pediculus humanus capitis*	*Steinernema rarum*	NOE	Doucet *et al.*, 2008
Coleoptera	Coccinelidae	*Eriopis connexa*	*S. rarum*	NOE	Doucet *et al.*, 2008
			S. feltiae	LCHOR	Doucet *et al.*, 2008
		Hippodamia convergens	*S. rarum*	NOE	Doucet *et al.*, 2008
			S. feltiae	LCHOR	Doucet *et al.*, 2008
	Chrysomelidae	*Chrysodina* sp.	*S. rarum*	NOE	Doucet *et al.*, 2008
			S. feltiae	LCHOR	Doucet *et al.*, 2008
	Curculionidae	*Rhigopsidius piercei*	*S. rarum*	ACAB, NOE	Doucet *et al.*, 2008
		Naupactus cinereidorsum	*S. rarum*	NOE	Doucet *et al.*, 2008
			S. feltiae	LCHOR	Doucet *et al.*, 2008
	Meloidae	*Epicauta adspersa*	*S. rarum*	NOE	Doucet *et al.*, 2008
			S. feltiae	LCHOR	Doucet *et al.*, 2008
	Melyridae	*Atylus astromaculatus*	*S. feltiae*	LCHOR	Doucet *et al.*, 2008
	Scarabaeidae	*Cyclocephalla signaticolis*	*S. carpocapsae*[a]		Stock, 1995
			S. feltiae[a]		Stock, 1995
		Diloboderus abderus	*S. carpocapsae*[a]		Stock, 1995
			S. feltiae[a]		Stock, 1995
	Tenebrionidae	*Tenebrio molitor*	*S. rarum*	ACAB, NOE, OLI	Doucet *et al.*, 2008
			S. feltiae	LCHOR	Doucet *et al.*, 2008
Diptera	Culicidae	*Aedes aegypti*	*S. rarum*	OLI	Doucet *et al.*, 2008
		Culex apicinus	*S. rarum*	OLI	Cagnolo and Almirón, 2010
		C. quinquefasciatus	*S. rarum*	OLI	Doucet *et al.*, 2008
		C. pipiens	*S. feltiae*	LCHOR	Doucet *et al.*, 2008
	Muscidae	*Musca domestica*	*S. rarum*	NOE	Doucet *et al.*, 2008
			S. feltiae	LCHOR	Doucet *et al.*, 2008
	Trypetidae	*Ceratitis capitata*	*S. rarum*	ACAB, NOE	Doucet *et al.*, 2008
			S. feltiae	LCHOR	Doucet *et al.*, 2008
Hemiptera	Aphidae	*Acyrthosiphon kondoi*	*S. rarum*	NOE	Doucet *et al.*, 2008
			S. feltiae	LCHOR	Doucet *et al.*, 2008
	Coreidae	*Pachylis argentinus*	*S. feltiae*	LCHOR	Doucet *et al.*, 2008
	Nabidae	*Nabis* sp.	*S. feltiae*	LCHOR	Doucet *et al.*, 2008
	Lecanidae	*Ceroplastes grandis*	*S. rarum*	NOE	Doucet *et al.*, 2008
			S. feltiae	LCHOR	Doucet *et al.*, 2008
	Pentatomidae	*Dichelops furcatus*	*S. rarum*	NOE	Doucet *et al.*, 2008
			S. feltiae	LCHOR	Doucet *et al.*, 2008
		Nezara viridula	*S. rarum*	OLI	Doucet *et al.*, 2008
	Reduviidae	*Dipetalogaster maximus*	*S. rarum*	NOE	Doucet *et al.*, 2008
Hymenoptera	Apidae	*Apis mellifera*	*S. rarum*	NOE	Doucet *et al.*, 2008

Table 16.5. Continued.

	Formicidae	*Acromyrmex lundi*	*S. rarum*	NOE	Doucet *et al*., 2008
Isopoda	Armadillidiidae	*Armadillidium vulgare*	*S. rarum*	OLI	Doucet *et al*., 2008
Lepidoptera	Noctuidae	*Anticarsia gemmatalis*	*S. feltiae*	LCHOR	Doucet *et al*., 2008
			S. rarum	NOE, OLI	Doucet *et al*., 2008; Gianfelici *et al*., 2014
		Spodoptera frugiperda	*S. feltiae*	LCHOR	Doucet *et al*., 2008
			S. rarum	NOE	Doucet *et al*., 2008
			S. diaprepesi	SRC	Caccia *et al*., 2014; Del Valle *et al*., 2014
		Rachiplusia nu	*S. rarum*	NOE	Doucet *et al*., 2008
			S. feltiae	LCHOR	Doucet *et al*., 2008
		Heliothis sp.	*S. feltiae*	LCHOR	Doucet *et al*., 2008
			S. rarum[a]	NOE	Doucet, 1986; Doucet *et al*., 2008
		Helicoverpa gelotopoeon	*S. diaprepesi*	SRC	Caccia *et al*., 2014
	Pieraustidae	*Laxostege bifidalis*	*S. rarum*	NOE	Doucet *et al*., 2008
	Pieridae	*Colias lesbia*	*S. rarum*	NOE	Doucet *et al*., 2008
			S. feltiae	LCHOR	Doucet *et al*., 2008
	Pyralidae	*Galleria mellonella*	*S. rarum*	NOE, OLI	Doucet *et al*., 2008
			S. carpocapsae	PAMPA	Stock, 1995
			S. scapterisci	COLON	Stock, 1995
			S. diaprepesi	SRC	Lax *et al*., 2011; Del Valle *et al*., 2014
			S. ritteri		de Doucet and Doucet, 1990
			S. feltiae	LCHOR, ISABEL	Doucet *et al*., 2008; Stock, 1995
		Diatraea saccharalis	*S. rarum*	NOE	Doucet *et al*., 2008
			S. feltiae	LCHOR	Doucet *et al*., 2008
	Tortricidae	*Crocidosema aporema*	*S. rarum*	OLI	Gianfelici *et al*., 2014
Orthoptera	Gryllotalpidae	*Scapteriscus borellii*	*S. scapterisci*[a]		Stock, 1995

Table 16.6. Spectrum of experimental and naturally infected susceptible hosts to entomopathogenic nematodes (Heterorhabditidae). (Adapted from Doucet *et al*., 2008).[a]

Host range			Nematode	Isolate	References
Anoplura	Pediculidae	*Pediculus humanus capitis*	*Heterorhabditis bacteriophora*	OLI	Doucet *et al*., 2008
Arachnida	Tetranychidae	*Panonichus ulmi*	*H. bacteriophora*	Nq, INTA A-V, INTA 11-12, ROCA	Giayetto and Cichón, 2006
Blattodea	Blattidae	*Blatta orientalis*	*H. bacteriophora*	RIV	Doucet *et al*., 2008
		Periplaneta americana	*H. bacteriophora*	RIV	Doucet *et al*., 2008
Coleoptera	Coccinelidae	*Hippodamia convergens*	*H. bacteriophora*	Nq	Giayetto and Cichón, 2006
	Chrysomelidae	*Diabrotica speciosa*	*H. bacteriophora*	RIV	Doucet *et al*., 2008
		Plagiodera erythroptera	*H. bacteriophora*	RIV	Doucet *et al*., 2008
		Xanthogaleruca luteola	*H. bacteriophora*	BIODIV., Nq, INTA A-V, INTA 11-12, ROCA	Giayetto and Cichón, 2006
	Curculionidae	*Pantomorus leucoloma*	*H. bacteriophora*	RIV	Doucet *et al*., 2008
		Rhigopsidius piercei	*H. bacteriophora*	ACAB	Doucet *et al*., 2008
		Naupactus xanthographus	*H. bacteriophora*	RIV	Doucet *et al*., 2008
		Graphognathus leucoloma	*Heterorhabditis argentinenins*[a]		Stock, 1993b
	Melyridae	*Atylus astromaculatus*	*H. bacteriophora*	RIV	Doucet *et al*., 2008
	Nitidulidae	*Lobiopa insularis*	*H. bacteriophora*	VELI	Achinelly *et al*., 2013
	Platypodidae	*Platypus sulcatus*	*H. bacteriophora*	BIODIV., Nq, INTA A-V, INTA 11-12, ROCA	Giayetto and Cichón, 2006
	Scarabaeidae	*Diloboderus abderus*	*H. bacteriophora*	VELI	Camino *et al*., 2013b
	Tenebrionidae	*Tenebrio molitor*	*H. bacteriophora*	VELI	Salas *et al*., 2013
Diptera	Culicidae	*Aedes aegypti*	*H. bacteriophora*		Peschiutta *et al*., 2014
	Trypetidae	*Ceratitis capitata*	*H. bacteriophora*	ACAB	Doucet *et al*., 2008
Hemiptera	Cercopidae	*Zulia entreriana*	*H. bacteriophora*	RIV	Doucet *et al*., 2008
	Cicadellidae	*Empoasca* sp.	*H. bacteriophora*	RIV	Doucet *et al*., 2008
		Edwarsiana crataegi	*H. bacteriophora*	BIODIV., INTA A-V, INTA 11-12	Giayetto and Cichón, 2006
	Margarodidae	*Icerya* sp.	*H. bacteriophora*	RIV	Doucet *et al*., 2008
	Membracidae	*Ceresa* sp.	*H. bacteriophora*	RIV	Doucet *et al*., 2008
	Pentatomidae	*Piezodorus guildinii*	*H. bacteriophora*	RIV	Doucet *et al*., 2008
	Reduviidae	*Triatoma infestans*	*H. bacteriophora*	RIV	Doucet *et al*., 2008
Hymenoptera	Apidae	*Apis mellifera*	*H. bacteriophora*	RIV	Doucet *et al*., 2008
	Formicidae	*Acromyrmex lundi*	*H. bacteriophora*	Biodiv., Nq, INTA A-V, INTA 11-12, ROCA	Giayetto and Cichón, 2006
Lepidoptera	Danaidae	*Diogas erippus*	*H. bacteriophora*	RIV	Doucet *et al*., 2008

Table 16.6. Continued.

	Nymphalidae	*Agraulis vanillae*	*H. bacteriophora*	RIV	Doucet *et al*., 2008
	Noctuidae	*Anticarsia gemmatalis*	*H. bacteriophora*	RIV, N4, N82	Doucet *et al*., 2008; Gianfelici *et al*., 2014
		Heliotis sp.	*H. bacteriophora*[a]	RIV	Agüera de Doucet and Doucet, 1986
		Spodoptera frugiperda	*H. bacteriophora*	RIV, VELI	Doucet *et al*., 2008; Camino *et al*., 2013b
		S. praefica	*H. bacteriophora*	RIV	Doucet *et al*., 2008
	Pieridae	*Colias lesbia*	*H. bacteriophora*	RIV	Doucet *et al*., 2008
	Pyralidae	*Galleria mellonella*	*H. bacteriophora*	BIODIV., VELI, RIV, OLI, RN, Nq, INTA A-V, INTA 11-12, ROCA	Achinelly *et al*., 2011; Giayetto and Cichón, 2006
	Psychidae	*Oiketicus kirbyi*	*H. bacteriophora*	RIV	Doucet *et al*., 2008
		O. platensis	*H. bacteriophora*	BIODIV., Nq	Giayetto and Cichón, 2006
	Saturniidae	*Automeris coresus*	*H. bacteriophora*	RIV	Doucet *et al*., 2008
		Rothschildia jacobaeae	*H. bacteriophora*	RIV	Doucet *et al*., 2008
		Hylesia nigricans	*H. bacteriophora*	VELI	Camino *et al*., 2013b
	Sphingidae	*Phlegetonthius sextapaphus*	*H. bacteriophora*	RIV	Doucet *et al*., 2008
	Tortricidae	*Crocidosema aporema*	*H. bacteriophora*	N4, N82	Gianfelici *et al*., 2014
		Cydia pomonella	*H. bacteriophora*	BIODIV., RIV, Nq, INTA A-V, INTA 11-12, ROCA	Giayetto and Cichón, 2006; Doucet *et al*., 2008
		Grapholita molesta	*H. bacteriophora*	BIODIV., Nq, INTA A-V, INTA 11-12, ROCA	Giayetto and Cichón, 2006
Orthoptera	Acridiidae	*Xyleus modestus*	*H. bacteriophora*	RIV	Doucet *et al*., 2008
Thysanoptera	Thripidae	*Frankliniella occidentalis*	*H. bacteriophora*	BIODIV., Nq, INTA 11-12, ROCA	Giayetto and Cichón, 2006

(Giayetto and Cichón, 2006). The susceptibility of the lepidopterans *C. aporema* and *A. gemmatalis* larvae to *H. bacteriophora* (N4 and N82 strains) was tested, indicating levels of parasitism higher than 75% at a dose of 50 IJs/insect (Gianfelici *et al.*, 2014).

H. bacteriophora (strain VELI) isolated from organic gardens constituted the second record of this species in the Buenos Aires province (Achinelly *et al.*, 2011). The Buenos Aires province is a principal area where farming is developed, and products are obtained for both domestic consumption and export. For this reason, investigations have been undertaken on the potential of this isolate as a biocontrol agent. The susceptibility to infection against lepidopterans produced over 80% parasitism in *Galleria mellonella*, *S. frugiperda* and *Hylesia nigricans*. This strain was characterized by a delay in mortality, unlike other *H. bacteriophora* strains from Argentina where nematodes killed the insects between 48 h (57.14%) and 72 h (42.85%) after contact (Camino *et al.*, 2013b; Salas *et al.*, 2013).

Efficacy was also determined against the generalist herbivore sap beetle, *Lobiopa insularis* (Coleoptera: Nitidulidae), which has recently become registered as an emerging pest in the cultivation of strawberries. Results demonstrated the susceptibility of that species to the native strain, thus pointing to a valuable and environmentally friendly alternative for the control of this beetle (Fig. 16.3). Mortality was higher in the larvae and pupae at 79 and 63%, respectively, than in adults at 10% (Achinelly *et al.*, 2013; Camino *et al.*, 2013b). The combination of cultural practices and the use of juveniles of the EPN *H. bacteriophora* in baited pitfall traps is being evaluated to control this strawberry sap beetle by the Centro de Estudios Parasitológicos y de Vectores (CEPAVE) in crops of the city of La Plata, Buenos Aires province.

16.5 Application of Entomopathogenic Nematodes in Vegetable Crop Protection

16.5.1 Field conditions

Although studies under laboratory conditions have been made in Argentina, the evaluation of EPNs under field conditions is only in the initial stages. *Steinernema riobravis* Cabanillas, Poinar and Raulston, 1994, Thermo Trilogy strain (Nematoda, Steinernematidae) was used for application to a small-scale field containing *Neocurtilla claraziana* (Saussure, 1874; Orthoptera, Gryllotalpidae), a pest of the soil in three gardens of Greater La Plata, Buenos Aires, Argentina (Camino and Reboredo, 2014). The highest dose (80,000 nematodes/3 m^2) produced a parasitism of 83%. Although high levels of infection were observed at all doses for the first 3 days post-treatment, effectiveness became significantly lower on day 7 and thereafter decreased progressively down to 0% on day 31.

16.5.2 Greenhouse environment

The effects of insect cadavers infected with three isolates of *H. bacteriophora* and one isolate of

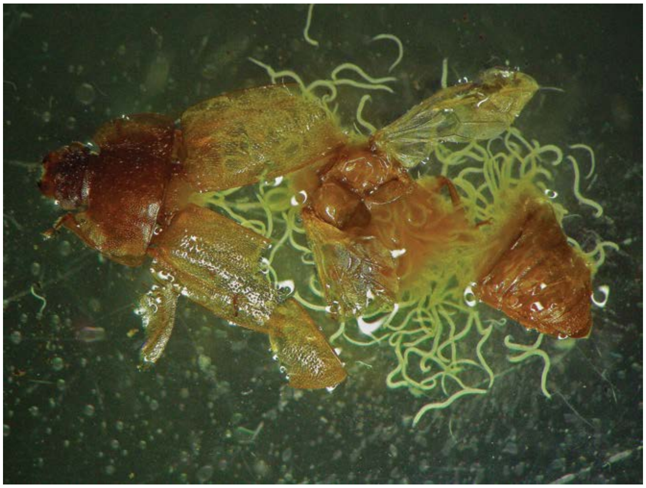

Fig. 16.3. Adult of the strawberry sap beetle, *Lobiopa insularis*, parasitized by the entomopathogenic nematode, *Heterorhabditis bacteriophora* strain VELI.

S. diaprepesi, were evaluated on a population of *Meloidogyne incognita* in pepper (*Capsicum annuum*) and summer squash (*Cucurbita maxima*) in greenhouse experiments carried out in Santa Fe (Argentina). Two 6-day-old insect cadavers per pot were placed below the soil surface, and the soil was inoculated with 100 second-stage juveniles of *M. incognita*.

Sixty days after inoculation, the following parameters were recorded for each plant: number of leaves; dry weight of aerial parts; and numbers of galls, egg masses and eggs.

In pepper, the only variable affected was the number of eggs, in treatments with the application of *Tenebrio molitor* cadavers infected with *H. bacteriophora* Rama Caída strain.

In summer squash, several treatments using infected cadavers resulted in a decrease in the numbers of galls and egg masses. Only the treatment involving *G. mellonella* cadavers infected with the *H. bacteriophora* Rama Caída strain proved to be efficient in reducing the number of *M. incognita* eggs (Del Valle *et al.*, 2013).

The effect of the application of two such native isolates – *S. rarum* from the province of Córdoba and *H. bacteriophora* from the province of Mendoza – to a *Nacobbus aberrans* population on tomato plants under greenhouse conditions was evaluated by Caccia *et al.* (2012). The inoculation of IJs (25 IJ/cm^2) on to the soil surface compromised the reproductive ability of the target nematode, causing a decrease in the subsequent reproduction of *N. aberrans* on the plants by 57 and 53% by the two biocontrol nematodes, respectively.

Even the use of *H. bacteriophora*-infected cadavers of *G. mellonella* caused a significant decrease in the number of galls and egg masses on pepper plants parasitized by *Meloidogne javanica*, in a growth chamber (Del Valle and Doucet, 2014). However, the use of EPNs does not always reduce plant parasitic nematode (PPN) populations, so the outcomes of their interactions vary with the EPN and PPN species, the host crop and the method used to evaluate the impact on PPNs.

16.6 Other Applications of Entomopathogenic Nematodes in Argentina

16.6.1 Entomopathogenic nematodes against Diptera (Culicidae, Muscidae)

Although Steinernematidae and Heterorhabditidae are terrestrial organisms that move in the interstitial water between soil particles, several authors have evaluated the aquatic habitat as an excellent environment for the survival of these nematodes. As was mentioned under Section 16.4.1, EPNs were tested against some insects of health importance. The infectivity and life cycle of an Argentine isolate of the nematode *H. bacteriophora* was registered on *Ae. aegypti* larvae under laboratory conditions (Peschiutta *et al.*, 2014). The higher percentages of parasitism, 77.5%, 78.3% and 84.2%, corresponded to the dose 1500:1, 500:1 and 750:1 IJs/larva, respectively. The mortality of mosquito larvae began to be registered 48 h after exposition to the nematodes. *H. bacteriophora* completed its life cycle within this host successfully up to the adult stage and the emergence of IJs in few days (3–8 days). The largest number of individuals was obtained with doses 100:1 (168.2 IJs/larva).

One generation of nematodes was observed in 70% of the parasitized larvae, while a second generation was observed in a variable percentage of larvae according to the dose.

Melanization was registered from a dose of 15:1 IJs/larva, in the abdomen (50.3%), thorax (39.0%), head (6.4%) and neck (4.3%) of the mosquito larvae. The number of melanized nematodes increased with higher doses.

Cagnolo and Almirón (2010) reported for the first time parasitism by *S. rarum* in larvae of *Cx. apicinus*. They exposed mosquito larvae at 1:1, 5:1, 10:1, 15:1, 100:1 and 400:1 IJs/larva, obtaining higher mortality at the major doses (75%). Although auspicious results were obtained, efficacy has not been tested on a large scale. The results demonstrated the presence of IJs in the offspring of the first- and second-generation adults, although with a greater virulence in the first-generation IJs. The authors considered that these generational differences might be exploited for biocontrol purposes through the use of a specific generation of IJs for inoculative releases.

16.7 Role of Entomopathogenic Nematodes in Integrated Pest Management in Argentina

IPM includes preventive tasks that should be applied in a continuous and organized way to minimize the hazards caused by the presence of economic pests. It is integrated by practices, tools and the control of materials, such as natural enemies of pests, crop rotation and the use of resistant crop varieties, not excluding the use of pesticides. Unlike traditional pest control (reagent system), IPM is a proactive system that anticipates the impact of the incidence of pests in production processes.

In Argentina, IPM began to take hold in the 1990s as a decision system based on the selection and use of tactical control, singly or combined harmoniously into a strategy related to analysis of the cost/benefit ratio. It is estimated that these practices have grown considerably in recent years, supported by increasingly important professional advice, which is directly related to the health of crops and the high yields achieved (SAGPyA, 2002).

16.7.1 Tasks include

Preventive methods

In order to reduce the chance of an attack: identify risk areas; choose a suitable site for planting; prepare or correct the soil; use pest-free plants; use good sanitary quality seeds and seedlings; choose cultivars well adapted to local conditions; select resistant or tolerant varieties.

Monitoring

Monitoring is a fundamental tool in deciding which strategy to follow.

For effective management, observation of the crop throughout the year should be made frequently, determining which insects are present, their abundance, stages and economic injury level.

These tasks also include maintenance and hygiene, since insect pests need environments that provide them with air, moisture, food or shelter. Some of these are removal from the production area of plants or severely infested plant organs and weeds that are pest hosts, which could affect the crops. The use of mechanical methods to remove insects or mites, remove caterpillars manually, use of high-pressure water to remove aphids or thrips, border crops, trap crops, pruning, irrigation, fertilization, harvesting time and frequency. For crops under cover, exclusion nets or other physical barriers, mainly for aphids and thrips, are used. While many herbs or their extracts are used to control insects, mites and other harmful organisms, these crops also have their own pests, so it should be important to control them.

16.7.2 Application of products

Chemicals or curative

Low toxicity when the pest exceeds the threshold of economic damage.

Biological: natural control, with conservation of natural enemies

Once you know the type of pest to be controlled, it is necessary to plan implementation of the products. The decision on the form of application and the equipment used is crucial to obtaining good results. All insecticide used must be registered with the National Health Service, SENASA.

Verification (management control)

This task is very important and works directly on an analysis of the evolution of IPM, and helps significantly in detecting the origin of the presence of pests. In Argentinean agriculture, it is common to find family subsistence models, many of which apply pesticides at random, depending not only on the presence of pests but also on the availability of resources and the cost of pesticides. This often generates an application process that is not coordinated with neighbours, is often done in adverse weather conditions and there is widespread ignorance of the phenology of crops and pest status, as well as the toxic effects of pesticides.

The application of bioinsecticides based on entomopathogenic control in Argentina includes mainly the use of bacteria (*B. thuringiensis*; *Bacillus subtilis*) and viruses (Betabaculovirus; Baculoviridae) to control lepidopteran pests of fruit and nut trees (Sciocco-Cap, 2013). The generation, production and introduction of biopesticides is considered a cost-effective alternative compared to the application of chemicals, especially for small organic farmers who often develop management systems with low budgets. Currently, there are no records of EPNs in the National Register of Biopesticides of the *Servicio Nacional de Sanidad* (SENASA), so the applications and checks carried out have been done with native agents on a small scale, mainly for research and development (Huerga and San Juan, 2005).

In this context, the introduction of a native isolate, the nematode *H. bacteriophora* VELI strain, to control the sap beetle, *L. insularis*, pest of strawberry crops in large areas of the provinces of Buenos Aires, Santa Fe and Corrientes, achieved good results by inoculation of IJs as the strategy. The cultivated area consisted of seven ridges divided into 15–16 plots each, each parcel with a total of 12 strawberry plants. The application was performed in six plots established at random, whose dose was 10,000 IJs/plant. The results suggested that

7 months post-introduction in the environment, IJs were spread almost evenly over the cultivated space, where nematodes were isolated from both plots (applied as well as non-applied). We considered that such distribution was influenced strongly by the irrigation system supplied to plants (D. Eliceche, La Plata, 2015, personal communication). Sap beetles are strongly attracted to certain volatile plant compounds in ripening or decaying fruit, and themselves produce pheromones/kairomones that elicit an aggregating behaviour. Baits using such material can be effective in trapping and monitoring sap beetle populations, and hence determining when treatment is necessary.

The application of IJs of the nematode *H. bacteriophora* along with fermented fruit in baited pitfall traps to control *L. insularis* could be an appropriate strategy, which is currently being tested. The introduction of *H. bacteriophora* VELI strain was performed in aubergine crops in organic farming in the province of Buenos Aires, to control the attack of the weevil, *Phyrdenus muriceus*. Nematodes introduced in an area of 1000 m^2 produced a reduction of the damage caused by the weevil at a rate of 51% when IJs were inoculated (2000 IJs/lineal metre) and 69% with inoculated cadavers (1 each 10 lineal metre) compared to the control (D. Eliceche, La Plata, 2015, personal communication). The knowledge and awareness of the producers of the benefits of the optimum time for application of a biopesticide, as is the early introduction of the nematode for pest control, is relevant. Early introduction of *H. bacteriophora* to control this pest prior to planting and/or postharvest would improve the performance and effectiveness of this biological agent, avoiding the attack of crops and the establishment of the insects.

The control of plant parasitic nematodes through the use of EPNs has recently been initiated in Argentina. As mentioned above, the strategy of applying insect cadavers infected with EPNs was tested by Del Valle *et al.* (2013) on the plant parasitic nematode *M. incognita* in pepper and summer squash, where treatments involving *T. molitor* and *G. mellonella* cadavers infected with the *H. bacteriophora* Rama Caída strain affected the number of *M. incognita* eggs produced on the pepper and summer squash, respectively. In the same way, Caccia *et al.* (2012) observed a reduction of the reproduction factor when evaluating the effects of isolates of *S. rarum* and *H. bacteriophora* against the phytophagus nematode *N. aberrans* in tomato plants.

IPM in Argentina has currently become not only a philosophy of control but also a reality that must be demonstrated, in an effort to ensure that the control of pests in a sustainable manner is achieved, obtaining quality products that are safe and environmentally friendly. The latter concept includes, as the main aim, the health of the farmer and his family, and the protection of natural resources.

16.8 Technical Restrictions

Bioproducts available in the market are scarce in Argentina and null for nematodes. This is due to the high production costs compared to chemical insecticides, lack of legislation regarding the regulation and registration requirements of different groups of bioproducts, the development of criteria or policy recommendations for field studies or releases of new bioproducts and the identification of mechanisms to promote the development, marketing and use of bioproducts by the producers, as well as the control of the collection, introduction, use and/or transit of such products.

However, recently the Advisory Committee on Bio-inputs for Agricultural Use (CABUA) has been created, in the area of the National Biotechnology Advisory Committee (NBAC), to provide technical advice on the quality, efficacy and biosafety requirements necessary in the use of agricultural bioproducts, as well as to establish an appropriate regulatory framework for their use, handling and disposal in the ecosystem. This constitutes an important stimulus for the growth of this line of research in Argentina, and increases awareness of the importance of the search, use and production of bioproducts, as well as the preparation of control plans, IPM and, likewise, the need for production at the national level.

16.9 Entomopathogenic Nematodes – Potential Threat to the Ecosystem?

Studies on the impact of EPNs on beneficial soil fauna are limited to the introduction in the field of a native strain of nematode *H. bacteriophora* (VELI) on beetles in strawberry crops. The insect survey pre- and post-inoculation showed no significant differences in the number and type of registered species. Parasitism and mortality by this nematode on beneficial insects were not observed in the field. In order of importance, individuals of Staphylinidae, Carabidae and Lathrididae families were collected (Camino *et al.*, 2013b).

16.10 Conclusion

A comprehensive study of entomonematodes has been carried out in Argentina, from the 1980s to the present. Researches were directed to surveys, diversity, identification, pathogenicity and life cycles, tending mostly to find new biocontrol tools.

Eight families and 43 genera of nematodes associated to insects were recorded, with different kinds of association (parasites, pathogens and parasitoids), three of which with a potential as biocontrol agents of insects (Mermithidae, Steinernematidae and Heterorhabditidae). A wide range of insects were recorded as hosts covering eleven orders and 44 families, most of agricultural and public health importance. Entomonematodes presented a wide distribution in a variety of environments: aquatic, terrestrial, arid and humid climates, cultivated and non-cultivated, plains, mountains, valleys, steppes and forests. Some highlights of the research included the study of the nematode *S. spiculatus* in the biocontrol of mosquitoes of public health importance, with research on optimizing production, the effect of abiotic and biotic factors on infectivity, and parasitic activity and population dynamics in the field. About 30 isolates of heterorhabditid and steinernematid nematodes have been cited by researchers from Argentina over more than 35 years of study. *S. rarum* and *S. ritteri* were described as new species for first time in Argentina. The pathogenicity of EPNs included laboratory and field studies on the susceptibility of pests from horticultural crops such as tomatoes, peppers, squash, aubergine and strawberries. The isolation and study of nine species of EPNs, namely *S. carpocapsae*, *S. feltiae*, *S. scapterisci*, *S. rarum*, *S. ritteri*, *S. glaseri*, *S. diaprepesi,* and *H. bacteriophora* and *H. argentinensis* (both synonymized by Adams *et al*., 1998), with different isolates, and records for the provinces of Córdoba, Neuquén, Río Negro, Santa Fe, La Pampa and Buenos Aires has been reported so far.

In Argentina, IPM has grown considerably in recent years, supported by increasingly important professional advice and directly related to the health of crops and the high yields achieved. The application of bioinsecticides based on entomopathogens for control currently includes the use of bacteria and viruses against lepidopteran pests of fruit and nut trees. The generation, production and introduction of biopesticides is considered a cost-effective alternative compared to the application of chemicals, especially for small organic farmers who frequently develop management systems on low budgets. So far, no company has formalized the registration of products based on EPNs in Argentina, with application and checks having only been carried out on a small scale with native agents, mainly for research and development. However, the use of nematodes in IPM, although incipient, has shown encouraging results, reducing the damage caused by pests of horticultural crops such as insects of the Coleoptera and Lepidoptera order and the root-knot nematodes, *N. aberrans*, *M. incognita* and *M. javanica*.

Although an extensive study has been conducted on entomonematodes in Argentina, the future of these investigations must involve further field trials and refinements in the techniques of mass production, along with long-term studies to ascertain the dynamics and development of these entomoparasites within natural populations of insects that are relevant to both agriculture and human health.

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