NATURAL POPULATIONS OF ENTOMOPATHOGENIC NEMATODES IN THE PAMPEAN REGION OF ARGENTINA

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ABSTRACT

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A survey of entomopathogenic nematodes was conducted in the Pampean region of Argentina. The objective was to identify wild isolates of entomopathogenic nematodes that are virulent to native insect pests. A total of 310 soil samples from 14 localities were tested for the presence of steinernematid and heterorhabditid nematodes by baiting with *Galleria mellonella* larvae. Additionally, 264 insects collected during the survey were examined for the presence of entomopathogenic nematodes. Entomopathogenic nematodes were recovered from 41 samples, 65.9% were steinernematids (*Steinernema feltiae, S. carpocapsae* and *S. scapterisci*) and 34.1% were heterorhabditids (*Heterorhabditis bacteriophora* and *H. argentinensis*). Loam soils yielded nematode populations on 19 occasions, and sandy loam soils on 22 occasions. No nematodes were found in clay soils. Entomopathogenic nematodes belonging to the families Steinernematidae and Heterorhabditidae were found in 27 of the 264 insects collected in the survey. These were recovered from alfalfa weevils (Coleoptera: Curculionidae), white grubs (Coleoptera: Scarabeidae) and mole cricket nymphs (Orthoptera: Gryllotalpidae).

Key words: Geographical distribution, entomopathogenic nematodes, Pampean region, Argentina, *Steinernema, S. carpocapsae, S. feltiae, S. scapterisci, Heterorhabditis, H. bacteriophora, H. argentinensis.*

RESUMEN

Stock, S. P. 1995. Poblaciones naturales de nematodos entomopatógenos en la región Pampeana de Argentina. Nematropica 25:143-148.

Un relevamiento de nematodos entomopatógenos fue realizado en la region Pampeana en la República Argentina con el objeto de aislar e identificar poblaciones autóctonas de estos parásitos cuya capacidad patogénica pueda ser evaluada frente a insectos plaga de dicha region. Un total de 310 muestras de suelo fueron extraídas de 14 localidades distintas. La extracción de nematodos se realizó mediante el empleo larvas de *Galleria mellonella* usadas como "insectos trampa", como así también de insectos colectados durante los muestreos. Nematodos pertenecientes a la familia Steinernematidae (*Steinernema feltiae, S. carpocapsae* y *S. scapterisci*) fueron hallados en un 65.9% de las muestras positivas, mientras que el 34.1% restante estuvo representado por nematodos de la familia Heterorhabditidae (*Heterorhabditis bacteriophora* y *H. argentinensis*). Sobre un total de 264 insectos colectados (Orden Coleoptera: Scarabeidae, Curculionidae; Orden Orthoptera: Gryllotalpidae), 27 de ellos fueron hallados parasitados naturalmente por steinernematidos y heterorhabditidos.

Palabras clave: Distribución geográfica, nematodos entomopatógenos, region Pampeana, Argentina, *Steinernema, S. carpocapsae, S. feltiae, S. scapterisci, Heterorhabditis, H. bacteriophora, H. argentinensis.*

INTRODUCTION

During the last 5 years, there has been heightened interest in Argentina concerning biological control of agricultural pests, particularly augmentative biological control of insects using entomopathogens. One of the most important agricultural

areas of Argentina is the Pampean region, which is subdivided into Humid and Semi-Arid Pampa subregions. Corn and alfalfa are important crops in this region, however, production has been declining in recent years (2). A percentage of the losses in both crops have been attributed to several insect pests such as alfalfa weevils (*Pantomorus-Naupactus* complex) in alfalfa and corn rootworm (*Diabrotica speciosa*) and white grubs (*Diloboderus* spp. and *Cyclocephala* spp.) in corn. These insects all have soil inhabiting life stages and are likely targets for control using entomopathogenic nematodes.

Entomopathogenic nematodes belonging to the families Steinernematidae and Heterorhabditidae meet many criteria for augmentative control of soil inhabiting insects through inundative releases (5). They have been applied successfully to control agricultural and horticultural insect pests in many countries, but in Argentina as in the rest of South America, they have not been fully exploited. One of the reasons is the lack of information about the identification and distribution of native nematodes which might allow for the selection of efficacious strains.

The objective of this study was to survey several corn and alfalfa fields, and native pastures from the Pampean Region of Argentina to isolate entomopathogenic nematodes which could be used as biological control agents against insect pests of these and other crops.

MATERIALS AND METHODS

Collection sites: A total of 310 soil samples was collected from 14 locations throughout the humid and semi-arid Pampean Region (Fig. 1). Soils were taken from 3 habitats: 1) corn fields, 2) alfalfa fields, and 3) permanent natural pastures from the surrounding areas of the corn and alfalfa fields. Within each habitat, samples were taken from 3-5 randomly selected sites. Each sample site was 10 m^2, and 5 pooled subsamples (10 $cm^2 \times 20$ cm deep) were collected with a small hand shovel. The subsamples were combined resulting in ca. 1 000 cm^3 of soil which were stored in polyethylene bags and placed in coolers (15-17°C) during transit to the laboratory. Samples were stored at 10°C ± 1°C until processed.

A total of 264 insects [135 alfalfa weevils (Coleoptera: Curculionidae), 95 white grubs (Coleoptera: Scarabeidae) and 34 mole cricket nymphs (Orthoptera: Gryllotalpidae)] encountered during the survey were collected and examined for nematode infection. The insects were placed individually in 100 cm^3 plastic containers with soil from the sampled area and transported to the laboratory.

Isolation of nematodes: From soil samples, presence of insect pathogenic nematodes was tested by baiting with wax moth larvae (*Galleria mellonella*) (3). Ten late-stage *G. mellonella* larvae were placed in the bottom of 500 cm^3 plastic containers which were then filled with sampled soil and ventilation holes added. The *Galleria* trap bioassays were incubated at 25°C, 85% relative humidity and kept in the dark. After 1 week, larvae were removed from the soil. Dead or moribund larvae were washed and either dissected and examined for presence of nematodes or placed on White traps to collect emerging infective-stage nematodes.

Dead insects collected during the survey were rinsed several times in water and then placed in White traps to collect emerging infective juveniles. Live insects were kept for 1 week in 100 cm^3 plastic containers and periodically observed for symptoms of parasitism of entomopathogenic nematodes. Nematodes collected from insect baits and natural hosts were

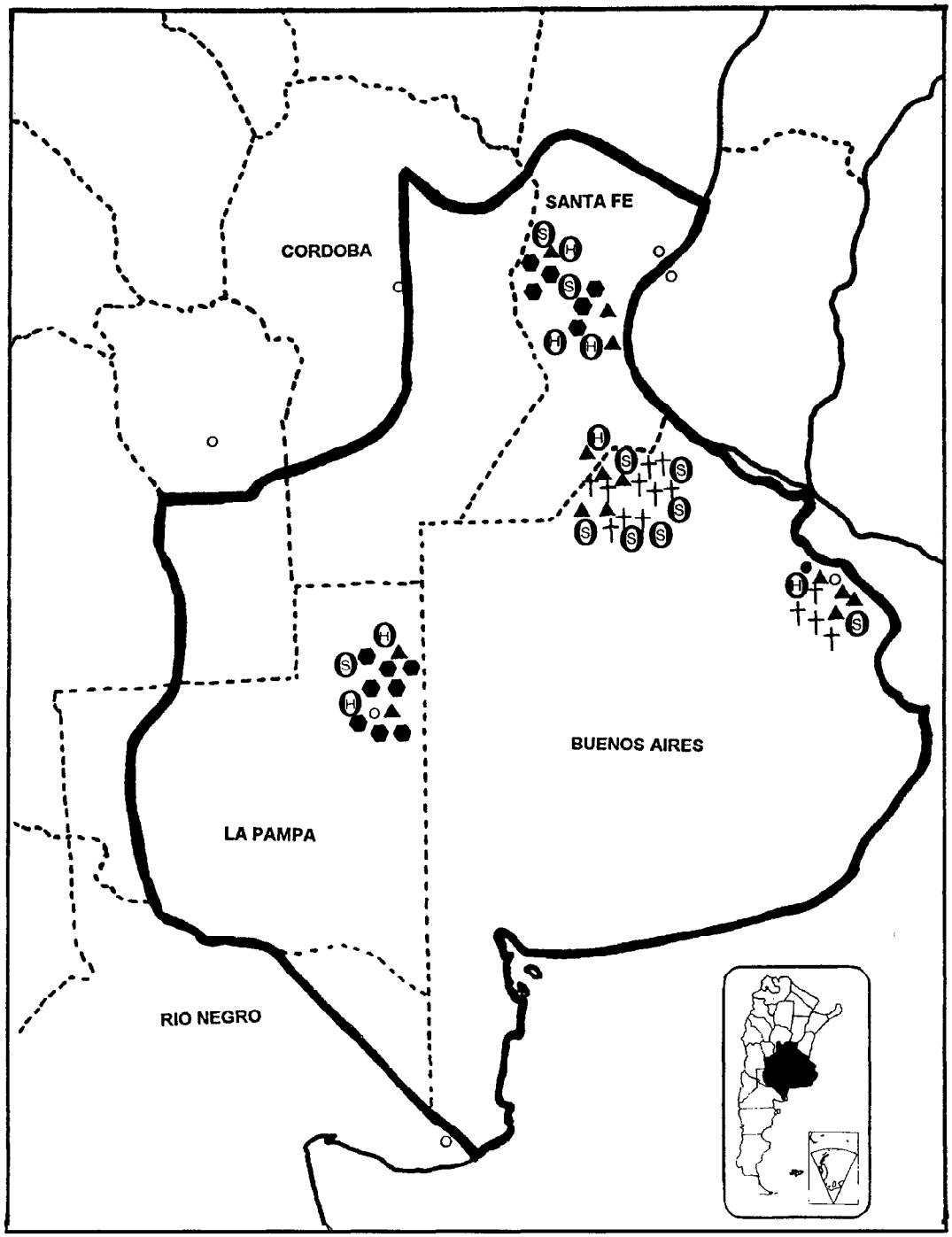

Fig. 1. Distribution of naturally occurring entomopathogenic nematodes in the Pampean region of Argentina. (H) *Heterorhabditis* and (S) *Steinernema* spp. in relation to corn (†), alfalfa (○) and natural pastures (Δ).

exposed to fresh *G. mellonella* larvae in Petri dishes to confirm pathogenicity and complete Koch's postulates.

Identification of nematodes: Nematodes recovered from either *Galleria mellonella* larvae or insects recovered during the survey were identified by microscopical examination using a Leitz Ortholux II microscope equipped with an ocular micrometer or Jandel[R] software video digitizer. The cross-hybridization technique of Poinar (10) was used to verify identity of *Steinernema* species.

RESULTS

Nematodes were isolated from both soil samples and insects collected during the survey. Entomopathogenic nematodes were recovered in 41 of the 310 soil samples (13.2%) and were found in all 3 habitats (Table 1). The most commonly isolated nematodes were steinernematids (65.9% of the positive samples). Morphological and morphometric studies together with the results obtained by the cross-mating experiments confirmed that the steinernematid nematodes isolated during the survey were *Steinernema feltiae* (Fillipjev) Isabel strain, *S. carpocapsae* (Weiser) Pampa strain, and *S. scapterisci* (Nguyen & Smar) Colon strain (Table 2). Heterorhabditid nematodes were recovered in 34.1% of the positive soil samples and were identified as *Heterorhabditis argentinensis* Stock and *H. bacteriophora* Poinar by comparative morphobiometrical studies. Loam soils (58.1% of total) yielded nematode populations in 19 samples and sandy loam soils (37.1% of total) yielded nematode populations in 22

Table 1. Location, soil texture and associated vegetation containing the entomopathogenic nematodes, *Steinernema* (S) and *Heterorhabditis* (H) spp., in the Pampean region of Argentina.

		No. samples with nematodes/No. samples tested[z]		
Location	Soil texture	Corn	Alfalfa	Pastures
Gorina	clay loam	0/0	0/0	0/15
La Plata	loam	0/0	0/0	0/15
Brandsen	loam	1(S)[a]/15	0/0	1(S)[a]/15
City Bell	loam	2(H)[d]/15	0/0	0/0
Salto	loam	2(S)[c]/15	0/0	1(S)[c]/15
Pergamino	loam	3(S)[b]/15	0/0	1(S)[b]/15
Colon	loam	0/0	0/0	1(S)[b]/15
Tres Sargentos	loam	0/0	0/0	1(S)[a]/15
Oliveros	loam	0/0	0/0	1(S)[a]/1(H)[c]/15
Rosario	loam	0/0	0/0	1(S)[a]/1(H)[d]/15
Rafaela	sandy loam	0/0	1(S)[b]/15	3(S)[a,b]/3(H)[d]/20
Santa Rosa	sandy loam	0/0	2(S)[a]/15	2(S)[a]/2(H)[c]/20
Anguil	sandy loam	0/0	1(H)[d]/15	1(S)[a]/1(H)[d]/15
Santa Fe	sandy loam	3S[a]/3H[c]/15	0/0	0/0
TOTAL SAMPLES		9S/5H/75	3S/1H/45	15/8H/190

[z]References: [a]*S. carpocapsae*; [b]*S. feltiae*; [c]*S. scapterisci*; [d]*H. bacteriophora*; [e]*H. argentinensis*.

Table 2. Results of cross-hybridization with steinernematid nematodes.[z]

Nematode strain	*S. carpocapsae* (NC strain)	*S. scapterisci* (Uruguay strain)	*S. feltiae* (SN strain)
Steinernema sp. (Isabel strain)	–	–	+
Steinerema sp. (Colon strain)	–	+	–
Steinernema sp. (Pampa strain)	+	–	–

[z]References: + = progeny; – = no progeny.

samples. No nematodes were found in clay loam soils (4.8% of total).

Entomopathogenic nematodes were found in 27 of the 264 insects collected in the survey (Table 3). Ten of 95 white grubs were parasitized by steinernematid nematodes (*Steinernema carpocapsae* and *S. feltiae*). The insects were identified as *Diloboderus abderus* and *Cyclocephala signaticolis* (Coleoptera, Scarabeidae), and the recovery of steinernematid species demonstrates these insects as natural hosts. The infected white grubs were recovered from Rafaela (province of Santa Fe), Salto and Brandsen (province of Buenos Aires). Another steinernematid nematode, *Steinernema scapterisci* was isolated from nymphs of mole cricket, *Scapteriscus borellii* (Orthoptera: Gryllotalpidae), at the locality of Colon (province of Buenos Aires). Seven of 34 nymphs were naturally parasitized by this nematode. *Heterorhabditis argentinensis* was recovered from alfalfa weevils, *Graphognathus leucoloma* (Coleoptera: Curculionidae), at the locality of Rafaela (province of Santa Fe). From a total of 135 weevils, 10 were parasitized by *H. argentinensis*. No natural host was found parasitized by *H. bacteriophora*, and this nematode was only isolated by baiting the soil samples with *G. mellonella* larvae.

DISCUSSION

The study was focused in the Pampean region because it is one the most important agricultural areas of Argentina. This investigation not only provides a collection of indigenous entomopathogenic nematodes but also a list of their natural hosts. Steinernematid and heterorhabditid nematodes were recovered mainly from natural pastures in the surrounding areas of corn and alfalfa fields. In contrast, Akhurst and Brooks (1), Griffin *et al.* (6) and Hominick and Briscoe (8) observed that entomo-

Table 3. List of natural hosts associated with entomopathogenic nematodes in Pampean region of Argentina.

Location	Insect Species	Number insects	Number parasitized	Nematode species
Rafaela	*Dilobederus abderus*	47	3	*Steinernema carpocapsae*
Salto	*Diloboderus abderus*		1	*Steinernema feltiae*
Brandsen	*Cyclocephala signaticolis*	48	2	*Steinernema carpocapsae*
Rafaela	*Cyclocephala sigaticolis*		4	*Sterinernema feltiae*
Rafaela	*Graphognathus leucoloma*	135	10	*Heterorhabditis argentinensis*
Colon	*Scapteriscus borelllii*	34	7	*Steinernema scapterisci*

pathogenic nematodes were more prevalent in agricultural fields than in natural habitats which differs with these results. However, present data demonstrate that even with cultural practices used, corn and alfalfa can sustain entomophilic nematode populations and that their release for biological control in these areas would provide an increased chance of nematode establishment.

Similar to other studies (4,6,7,10), nematode recovery in this survey was greater in sandy loam soil than in loam or clay loam soil. Steinernematids were recovered in both soil types whereas heterorhabditids were recovered predominantly from sandy loam soils. According to Wallace (11), loam soils have small pores which physically restrict nematode movement. However, reduced numbers found in clay soils also may be the result of low nematode recovery.

Choo *et al.* (4) indicated surveys are important because they document the occurrence of these nematodes in various habitats and localities and are also a source of new species and isolates. At the same time, the advantage of using native isolates of entomopathogenic nematodes for control is the likelihood of enhanced ecological compatibility and also in the reduction of significant impact on non-target organisms when compared with exotic isolates.

Accordingly, the isolated steinernematids and heterorhabditids will be evaluated in the future for their control potential against natural hosts under local conditions.

LITERATURE CITED

1. AKHURST, R. J, and W. M. BROOKS. 1984. The distribution of entomophilic nematodes (Heterorhabditidae and Steinernematidae) in North Carolina. Journal of Invertebrate Pathology 44:140-145.
2. BARIGGI, C., V. L. MARBLE, C. D. ITRIA, and J. M. BRUN. 1986. Investigación, tecnología y producción de alfalfa. INTA Colección Científica 22, 488 pp.
3. BEDDING, R. A., and R. J. AKHURST. 1975. A simple technique for the detection of insect parasitic rhabditid nematodes in soil. Nematologica 21:109-110.
4. CHOO, J. Y., H. K. KAYA, and S. P. STOCK. 1995. Isolation of entomopathogenic nematodes (Steinernematidae and Heterorhabditidae) from Korea. Japanese Journal of Nematology 25: (In press).
5. EHLER, L. E. 1990. Some contemporary issues in biological control of insects and their relevance to the use of entomopathogenic nematodes. Pp. 1-12 *in* R. Gaugler and H. K. Kaya, eds. Entomopathogenic Nematodes in Biological Control. CRC Press, Boca Raton, Florida.
6. GRIFFIN, C. T., J. F. MOORE, and M. J. DOWNES. 1991. Occurrence of insect-parasitic nematodes (Steinernematidae, Hetelorhabditidae) in the Republic of Ireland. Nematologica 37:92-100.
7. HARA, A. H., R. GAUGLER, H. K. KAYA, and L. M. LEBECK. 1991. Natural populations of entomopathogenic nematodes (Rhabditida: Steinernematidae and Heterorhabditidae) from the Hawaiian Islands. Environmental Entomology 20:211-216.
8. HOMINICK, W. M., and B. R. BRISCOE. 1990. Occurrence of entomopathogenic nematodes (Rhabditida: Steinernematidae and Heterorhabditidae) in British soils. Parasitology 100:295-302.
9. LIU, J., and R. E. BERRY. 1995. Natural distribution of entomopathogenic nematodes (Rhabditidae: Heterorhabditidae and Steinernematidae) in Oregon soils. Environmental Entomology 24:159-163.
10. POINAR, G. O. JR. 1975. Entomogenous Nematodes. A Manual and Host List of Insect-Nematode Associations. E. J. Brill, Leiden, The Netherlands, 317 pp.
11. WALLACE, H. R. 1958. Movement of eelworms. Annals of applied Biology 46:74-85.

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