Biodiversity of insect-parasitic nematodes in soil pest insect (Orthoptera, Gryllidae and Gryllotalpidae) in wheat fields of Buenos Aires, Argentina

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Resumen

Biodiversidad de nemátodos parásitos de insectos plaga del suelo (Orthoptera, Gryllidae y Gryllotalpidae) en áreas agrícolas de Buenos Aires, Argentina

El presente trabajo se llevó a cabo en campos de trigo de la región pampeana, Argentina, proporcionando una amplia lista de nemátodos parásitos de insectos plaga del suelo. Los adultos y ninfas de grillos y grillotopo, se encontraron parasitados por veinticuatro especies de nemátodos pertenecientes a seis familias. El grupo de nemátodos más numeroso fue el de los thelastomátidos, mientras que las familias Pseudonimidae, Travassosinematidae y Diplogasteridae fueron las menos representadas. La región pampeana constituye un área con alta diversidad de especies de nemátodos de insectos, muchas de las cuales podrían ser consideradas agentes biorreguladores de insectos plaga en zonas agrícolas de la Argentina.

Palabras clave: Nemátodos, Parasitismo, Plagas agrícolas, Orthoptera, Region Pampeana.

Abstract

This work was conducted in wheat fields of the Argentine pampas providing an extensive list of nematodes of soil pest insects. Adults and nymphs of crickets and mole crickets, were found parasited by nematodes. Twenty-four nematode species from six families were registered. Thelastomatid nematodes were the most numerous group while Pseudonimidae, Travassosinematidae and Diplogasteridae families were the least represented. The Pampean region constituted an area with high diversity of nematodes associated with insects and some of them, could be considered as biocontrol agents of troublesome insect pests within the agricultural areas of Argentina.

Key words: Nematodes, Parasitism, Agricultural pests, Orthoptera, Pampean Region.

Introduction

Interest in the biology and ecology of host-parasite relationships has increased in recent years. Many investigators have recognized that the parasites of insects have the potential of regulating

their host populations and communities (Poulin 1998). Regardless of their possible negative effects, such parasites may also be used as biological agents in various ways (Gardner & Campbell 1992). Within this context, the study of the host-parasite relationships could in many situations illustrate the close relationship between the phenomena of coevolution and cospeciation. Moreover, the use of parasites and pathogens is one promising approach in comprehensive pest management; where they are implemented. The benefits of biological agents include the economy of the production methods, the high insect mortality rates, the typical ability to adapt to new environments, and the feasibility of reaching the commercial market as a profitable biopesticide. Entomogenous nematodes are a large and extensive group among the insects' natural enemies (Hazir et al. 2004). Most of these parasites can either kill their hosts or at least cause sterilization; a reduction of fecundity; a decrease in longevity; a reduced flight capability; a delayed development; and adverse behavioural, physiological, and morphological changes within a wide range of insects (Poinar 1979). Associated with insects, there are approximately 30 nematode families, whose relationship ranges from merely fortuitous to frankly parasitic and pathogenic. In Argentina the knowledge of parasitic nematodes and other insect pathogens is scanty. Studies to date have been concerned with only the taxonomy and biology of certain species (Stock 1995, Camino & De Villalobos 1996, Doucet & Bertolotti 1996, Doucet & Laumond 1996, Camino et al. 1997, Camino & Szathmary 2001). Little data has been published on parasite diversity, and infestation percentage. The Pampean region is an important area where farming is developed and products are obtained for both domestic consumption and for export. Some of the most important pests are mole crickets and crickets, which constitute serious problems in crop soils because of their eating habits. Finding solutions are necessary for minimizing the risk by the action of the pest itself or by improper use of chemicals to control them.

The aim of this work was therefore to characterize the populations of nematode parasites infecting soil pest insects within the Pampean region of Argentina and to evaluate the ecological and diversity indices as indicators of the status of the nematode communities.

Materials and methods

A survey was carried out in different locations of the Pampean region, Buenos Aires province, Argentina (Fig. 1) over 10 years. A total of 180 soil random samples were collected from 7 locations (26 for six locations and 24 from Magdalena) in wheat fields (Table 2). Samples were taken from randomly selected sites with a shovel. The insects were collected in the field by standard methods (digging holes of 10 cm x 10 cm x 10 cm) and transported to the laboratory for exploration in plastic jars at room temperature. Adults and nymphs of *Gryllodes laplatae* (Saussure, 1874) (Gryllidae) (n=50), and *Neocurtilla claraziana* (Saussure, 1874) (Gryllotalpidae) (n=50), were collected from each locations. The insect hosts were kept at 5°C for 10 min and then dissected in Petri dishes filled with distilled water under a stereoscopic microscope. A transverse incision was made along the posterior end of the abdomen

Figura 1. Mapa de Sudamérica, provincia de Buenos Aires (Argentina) y detalle del área de muestreo.

Figure 1. Map of South America, Buenos Aires province and (Argentine) and detail map of the area sampled.

1. Lincoln; 2. Brandsen; 3. Gorina; 4,5,6 Gran La Plata (City Bell, Gonnet, Tolosa); 7. Magdalena.

and the digestive tract removed to observe the presence of the parasites in the intestine and body cavity. The latter were next transferred to a fixative of 50% (v/v) aqueous triethanolamine formalin, for 48 h and then placed in 100% triethanolamine formalin before transfer to glycerol for slow evaporation in order to clear the parasites (Seinhorst 1959). The fixed specimens were used for taxonomic identification following the key of Poinar (1975). Representative specimens were deposited at the Helminthological Collection, Museo de La Plata, Argentina.

The number of insects parasitized and the total insects collected were considered to obtain the percentage of parasitism. Some indices and parameters were calculated for each group of hosts: mole crickets and crickets (Table 1) (Bush et al. 1997)

Parameter	Formula
S: Species richness	Total amount of species
H: Shannon index	$-\sum_{i=1}^{S} p_i \ln(p_i)$ p_i= proportion of each species in a sample
D: Simpson Index	$\sum_{i=1}^{S} n_i(n_i-1)/N(N-1)$ N= total of individuals of all species. n= total number of individuals of each species
Q: Sørensen Index	$2C/(A+B)$ A, B= number of species in samples A and B, respectively C=number of species shared between two samples
E: Uniformity Index	D/S D: Simpson Index S: Species richness
MA: Mean abundance	Total of individuals of a parasite species in a sample of a host, divided by the total of hosts of that species examined (including both infected and uninfected hosts)
P: Prevalence	Hosts infected with a particular parasite species as a percentage of hosts examined for that same parasite
Intensity	Nematodes per insect over the total of parasitized hosts
Dominance	Nematodes per insect over the total number of hosts

Tabla 1. Índices y parámetros empleados.
Table 1. Indices and parameters calculated.

Results

Twenty-four nematode species belonging to 6 families were registered at 7 locations: Mermithidae, Thelastomatidae, Pseudonimidae, Travassonematidae, Diplogasteridae, and Rhabditidae (Table 2). Table 3 shows the number of specimens collected. The locations of Gorina and Lincoln exhibited a large number of species. The thelastomatid nematodes were the most numerous group with a total of 15 species registered among all the insects (Table 3). The Pseudonimidae, Travassosinematidae and Diplogasteridae families were the least represented (Fig. 2).

In *G. laplatae*, 16 nematode species were found belonging to 4 families (Table 2). The family Thelastomatidae constituted 53% of the total nematode species found (Fig. 2). However, the highest total number of parasites was registered in rhabditid nematodes (Table 3). *Mikoletzkya sp.* was the only specie for diplogasterid nematodes, Three species of the family Rhabditidae were present (Table 2). Family Mermithidae constituting 6.25% of the nematode species registered (Fig. 2). The percentage of parasitism for all nematode species varied between 16% and 80% (Table 3). High levels of parasitism and abundance were achieved for the rhabditid nematodes *Cruznema lincolnensis* Reboredo & Camino 1998 and *Alloionema sp.* The mean abundance was between 0.4 and 838 and dominance between 0.02% and 35%. The number of nematodes per larva registered ranged from 1.0 to 4,900; the greatest number was measured for *Pelodera sp.* (Rhabditidae) (Table 4). The values of species richness was S=16. The values for diversity were H=1.18 and D=0.34. The uniformity index was 0.021.

In *N. claraziana* (Fig. 2), a low number of nematode species (9) was registered. Thelastomatids nematodes constituted 62% of the total nematode species found (Fig. 2). Family Pseudonimidae included only one species, *Pseudonymus terrestris* Camino & Reboredo 2001. As well as Travassosinematidae, *Binema bonaerensis* Camino & Reboredo 1999 was the only species found. Family Mermithidae constituting 12.5% of the nematode species registered (Fig. 2). The percentage of parasitism for all nematode species varied between 28% and 60%. The mean abundance was between 0.4 and 1.8. The dominance ranged from

Parasites	Hosts	Location
Mermithidae		
Agamermis decaudata	*N. claraziana*	Magdalena
Amphimermis sp.	*N. claraziana, G. laplatae*	Brandsen
Hexamermis macrostoma	*G. laplatae*	City Bell
Thelastomatidae		
Blatticolla cristovata	*G. laplatae*	Tolosa
Cameronia laplatae	*G. laplatae*	City Bell
Cephalobellus lobulata	*N. claraziana*	Gorina
Cephalobium bidentata	*G. laplatae*	City Bell
Cephalobium dispar	*G. laplatae*	Gorina
Cephalobium laplata	*G. laplatae*	City Bell
Cephalobium magdalensis	*G. laplatae*	Magdalena
Cephalobium odontolateralis	*G. laplatae*	Gonnet
Cephalobium polidentatum	*G. laplatae*	Lincoln
Cephalobium tridentata	*G. laplatae*	Gorina
Euryconema brevicauda	*N. claraziana*	Gorina
Fontanema gracilis	*N. claraziana*	Gorina
Grillophila cephalobulata	*N. claraziana*	Gorina
Neyraiella distinctus	*G. laplatae*	City Bell
Scwenkiella tetradentatum	*N. claraziana*	Gorina
Pseudonymidae		
Pseudonymus terrestris	*N. claraziana*	Lincoln
Travassosinematidae		
Binema bonaerensis	*N. claraziana*	Gorina
Diplogasteridae		
Mikoletzkya sp.	*G. laplatae*	Lincoln
Rhabditidae		
Alloionema sp.	*G. laplatae*	Lincoln
Pelodera sp.	*G. laplatae*	Gonnet
Cruznema lincolnensis	*G. laplatae*	Lincoln

Tabla 2. Localización de los nemátodos y especies hospedadoras.
Table 2. Location of the nematodes and host species

8% to 28%. The number of nematodes per larva registered varied between 1.1 and 3.0 (Table 4). The value of species richness (S) was S = 9. The values of diversity were H=3.31 and D=0.002.

The value of Sorensen index was low, (0.08) with only one shared species (*Amphimermis sp.*). The uniformity index was 0.00025.

Discussion

This work provided an extended list of indigenous nematode parasites of two orthopteran pests found in soil of wheat fields of the Pampean region of Argentina. Twenty-four nematode species belonging to 6 families were recorded. The locations of Gorina and Lincoln, exhibited a greater diversity with 5 nematode species being present in those areas, respectively. The differences registered for the diversity of nematode species between locations could be related to abiotic factors such us temperature, humidity and pH of the soil, which not were determined in the present study. The highest degree of parasite-species richness within a given host was reached in *G. laplatae* with 16 nematode species recorded. The mole crickets, which are found in a common environment with crickets were parasitized by a lower number of species. A low numbers of mermitids were registered with two species.

Thelastomathid nematodes were widely represented in both hosts (62.5%). Similar conditions could obtain with those species of rhabditids, which group showed high values of abundance: i.e., *Cruznema lincolnensis*, *Alloionema sp.* and *Pelodera sp.* This last species was the most abundant but not showed high parasitism.

Jex et al. (2006) observed that species belonging to the Pseudonimidae family more frequently infect coleopterans. The presence of one species of Pseudoniminae in the mole cricket *N. claraziana* as host constitute the first record within the order Ortopthera. In agreement with the findings of Adamson & van Waerebeke (1992), we have recorded lower levels of specificity for the thelastomatid nematodes, which were capable of parasitizing both host species. The travassonematid nematodes are more fastidious and infect exclusively mole crickets (Jex et al. 2006). In this study we found one species in the mole cricket *N. claraziana*.

The Shannon Index is commonly used to assess species diversity, combining the parameters of evenness and richness; but this index may be influenced by the abundance or overall number of taxa. This characteristic could explain the higher indices registered in mole crickets, where no species was observed with a high dominance. Accordingly, with this we registered a high value in the uniformity index but a low one in the Simpson

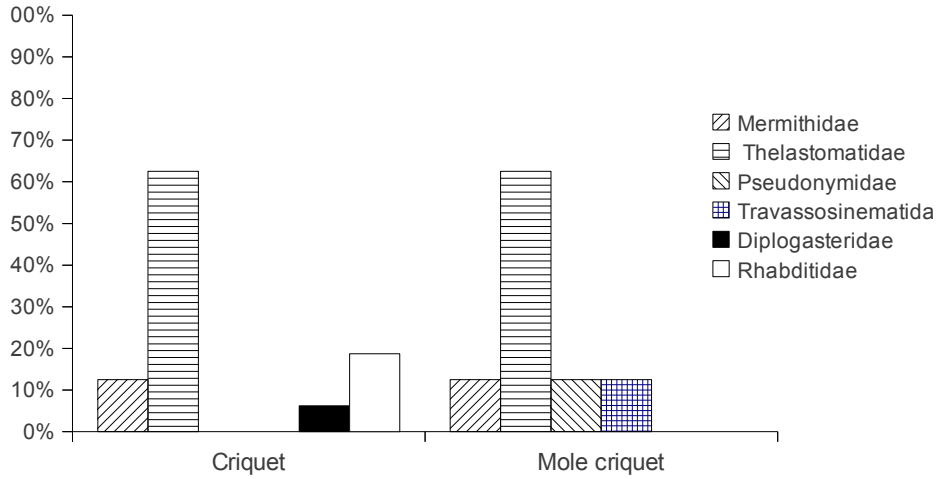

Figura 2. Porcentaje del número de especies pertenecientes a las diferentes familias de nemátodos de cada hospedador.
Figure 2. Percentage of number of species belonging to different families of nematodes for each host.

index, where the latter is an expression of the dominance of a given nematode species. Crickets harbor nematode species at higher dominances, and also showed higher Simpson-index values. The low values obtained for the Sorensen coefficient it comparing populations of nematode species between both hosts could be the result of a high host specificity on the part of the parasites.

Most of the nematode species constitute a significant regulatory influence on the population dynamics of these insect pests (Poinar 1979).

The Pampean region of Argentina constituted an area with high diversity of nematodes parasites of mole crickets, and crickets which some of them would be considered as biocontrol agent against troublesome insect pests within the agricultural areas.

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	Host species	
	Gryllidae	Gryllotalpidae
Parasites	***G. laplatae***	***N. claraziana***
Mermithidae		
Agamermis decaudata	-	90
Amphimermis sp.	20	-
Hexamermis macrostoma	20	-
Thelastomatidae		
Blatticolla cristovata	68	-
Cameronia laplatae	27	-
Cephalobellus lobulata	-	39
Cephalobium bidentata	48	-
Cephalobium dispar	39	-
Cephalobium laplata	41	-
Cephalobium magdalensis	37	-
Cephalobium odontolateralis	42	-
Cephalobium polidentatum	42	-
Cephalobium tridentata	25	-
Euryconema brevicauda	-	38
Fontanema gracilis	-	42
Grillophila cephalobulata	-	27
Neyraiella distinctus	35	-
Scwenkiella tetradentatum	-	31
Pseudonymidae		
Pseudonymus terrestris	-	39
Travassosinematidae		
Binema bonaerensis	-	38
Diplogasteridae		
Mikoletzkya sp.	47	-
Rhabditidae		
Alloionema sp.	37500	-
Pelodera sp.	39500	-
Cruznema lincolnensis	41900	-

Tabla 3. Número total de parásitos registrados por cada especie hospedadora (N= 50).
Table 3. Total number of parasites registered of every host species (N= 50).

	Host species							
	Gryllidae				Gryllotalpidae			
	G. laplatae				*N. claraziana*			
Parasites	***P***	***I***	***A***	***D***	***P***	***I***	***A***	***D***
Mermithidae								
Agamermis decaudata	-	-	-	-	60	3.0	1.8	28
Amphimermis sp.	36	1.1	0.4	0.02	-	-	-	-
Hexamermis macrostoma	36	1.1	0.4	0.02	-	-	-	-
Thelastomatidae								
Blatticolla cristovata	28	2.6	1.4	0.06	-	-	-	-
Cameronia laplatae	36	1.5	0.54	0.02	-	-	-	-
Cephalobellus cyclocephalae	-	-	-	-	50	1.6	0.8	12
Cephalobium bidentata	24	2.0	0.96	0.04	-	-	-	-
Cephalobium dispar	24	1.62	0.84	0.03	-	-	-	-
Cephalobium laplata	24	3.4	0.82	0.03	-	-	-	-
Cephalobium magdalensis	24	1.54	0.74	0.03	-	-	-	-
Cephalobium odontolateralis	24	1.75	0.84	0.03	-	-	-	-
Cephalobium tridentata	24	1.04	0.5	0.02	-	-	-	-
Euryconema brevicauda	-	-	-	-	32	1.18	0.8	12
Fontanema gracilis	-	-	-	-	65	1.3	0.4	13
Grillophila cephalobulata	-	-	-	-	28	1.92	0.5	8
Neyraiella distinctus	32	16	0.7	0.03	-	-	-	-
Scwenkiella tetradentatum	-	-	-	-	31	1.93	0.6	9
Pseudonymidae								
Pseudonymus terrestris	-	-	-	-	35	1.11	0.8	12
Travassosinematidae								
Binema bonaerensis	-	-	-	-	50	1.5	0.8	12
Diplogasteridae								
Mikoletzkya sp.	22	4.3	0.94	0.04	-	-	-	-
Rhabditidae								
Alloionema sp.	80	937	750	31	-	-	-	-
Pelodera sp.	16	4900	790	33	-	-	-	-
Cruznema lincolnensis	80	1047	838	35	-	-	-	-

Tabla 4. Prevalencia (P), número de nemátodos por larva (I), abundancia media (A), y dominancia (D) de los nemátodos parásitos registrados en plagas del suelo de la región Pampeana, Argentina.

Table 4. Prevalence (P), number of nematodes per larva (I), mean abundance (A), and dominance (D) of the nematode parasites registered from soil pests in Pampean region, Argentina.

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