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Short communication

Phoresy of the entomopathogenic nematode *Steinernema feltiae* by the earthworm *Eisenia fetida*

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Abstract

The free-living stage of entomopathogenic nematodes occurs in soil, and is an environmental-friendly alternative for biological control. However, their dispersal capability is limited. Earthworms improve soil characteristics, changing soil structure and influencing many edaphic organisms. Thus, earthworms could be used as vectors to introduce/disperse beneficial organisms. Nevertheless this interaction has not been studied in detail. This study presents the infectivity results of *Steinernema feltiae* after passing through the *Eisenia fetida* gut. Although entomopathogenic nematodes have no deleterious effects on earthworms, their passage through *E. fetida* gut seriously affected their mobility and virulence.

Keywords: *Eisenia fetida*; *Spodoptera littoralis*; *Steinernema feltiae*; Dispersal; Earthworm; Entomopathogenic nematodes; Phoretic association

1. Introduction

Entomopathogenic nematodes (EPNs) are an environmental friendly alternative for insect pest control; however their dispersal capability is limited (Kaya, 1990; Kaya and Gaugler, 1993). Earthworms improve soil conditions (aeration, drainage and organic matter content) and they are able to change soil structure, move large amounts of soil and affect microfloral and faunal diversity (Brown, 1995; Doube and Brown, 1998). Many associations (phoretic, paratenic intermediate or sole host) between nematodes and earthworms had been reported (Gunnardson and Rundgren, 1986; Poinar, 1978; Timper and Davies, 2004), and some authors think that earthworms could be used as vectors to introduce and/or disperse beneficial organisms (Eng et al., 2005; Shapiro et al., 1993, 1995). The aim of this study was to determine the pathogenicity of the entomopathogenic nematode *Steinernema feltiae* (Filipejv) Wouts, Mráček, Gerdin and Bedding (Rhabditida: Steinernematidae) against epigeic earthworm *Eisenia fetida* Savigny (Oligochaeta: Lumbricidae) and to study whether *S. feltiae* infective juveniles are virulent against Egyptian cotton leafworm *Spodoptera littoralis* Boisd. (Lepidoptera: Noctuidae) after their passage through *E. fetida* gut.

2. Materials and methods

2.1. Entomopathogenic nematodes, insects and earthworms

Nematodes, insects, and earthworms used in the experiment were reared under laboratory conditions. The native entomopathogenic nematode *S. feltiae* RIOJA strain was morphologically, molecularly and biologically characterized in *Galleria mellonella* L. (Lepidoptera: Pyralidae) (Campos-Herrera et al., in press). Commercial *S. feltiae* strain was supplied by Koppert Biological Systems (ENTONEM®). Both strains were cultured in vivo on *G. mellonella* larvae following the Woodring and Kaya method (1988), and the infective juveniles (IJs) suspension

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was cleaned, stored, and concentration adjusted according to the method of Glazer and Lewis (2000) prior to assay.

The insect *S. littoralis* was reared on an artificial diet (Poitout and Bues, 1974). Last instars larvae of 24 h were used in the bioassays. The earthworm *E. fetida* was obtained from a stock culture of Department of Zoology and Physical Anthropology, Faculty of Biological Sciences (Universidad Complutense from Madrid, Spain), and reared on a soil and horse manure mixture. Adult and young individuals were used in the bioassay. The insects and the earthworms were maintained at 22 ± 2 °C and 16:8 h, L:D.

2.2. Substrates

The greenhouse soil used in the earthworm bioassay was sieved (2 mm) and steam sterilized prior to bioassay. The organic matter content was 35% and pH 6.53. The virulence of the entomopathogenic nematodes alive post-exposure of the earthworm gut was tested on sterilized silica sand (particle size 0.16–1.6 mm).

2.3. Experimental procedures

To collect the intestinal content before the assays, adult and young individuals of *E. fetida* were rinsed in tap water and placed on damp filter paper in 9 cm diameter Petri dishes (10 individuals/dish) for 24 h at 18 °C (Hartenstein et al., 1981). The virulence of native and commercial EPNs against *S. littoralis* and *E. fetida* were carried out adding 2500 IJs/cm^2 on 10 g of sterilized greenhouse soil substrate in a 5-cm diameter Petri dish. The assay consisted on 20 *S. littoralis* larvae, 10 young and 20 adult individuals of *E. fetida* (two individuals per dish), using 25 Petri dishes without nematodes as control. The assay was incubated at 22 ± 2 °C for 24 h. After nematode treatment, the test organisms were carefully rinsed in mQ-water (Milli-Q Water System, Millipore S.A., Molsheim, France). *S. littoralis* larvae were individually transferred to Petri dishes with an artificial diet. The earthworms were individually transferred to 4 cm × 1 cm diameter tubes containing 500 μl mQ-water to obtain the first 24 h cast (cast I). Later, earthworms were again transferred to a new tube to obtain the second 24 h cast (cast II), and finally were transferred to Petri dishes with damp filter paper. The nematode transmission through the earthworm gut was assessed as an accumulative percentage of earthworms releasing IJs in their casts. The total number and percentage of mobile IJs in casts I and II were also recorded. The nematode virulence after passing through the earthworm gut was assessed by adding 1.3 g silica sand to the cast solution tubes and one *S. littoralis* larvae. The assay was incubated at 22 ± 2 °C for 10 days to record *S. littoralis* and earthworm mortalities, repeating the experiment three times.

2.4. Statistical analysis

ANOVA analysis was performed to observe differences within the assays. The percentages of *E. fetida* and *S. littoralis* mortalities were corrected by the method of Abbott (1925). The mortality and transmitting individual percentages were arcsine transformed before statistical analysis and compared using a Chi-Squared test. The mean value of number of IJs in cast was compared using a Mann–Witney test. Statistical analyses were performed by SPSS 12.0 for Windows and a significant level of $p \leqslant 0.05$ was used.

3. Results and discussion

3.1. *S. feltiae* virulence against *E. fetida* and *S. littoralis*

Steinernema feltiae were not pathogenic to *E. fetida*. Although the biological bases of non-susceptibility of earthworms to entomopathogenic nematodes are scarcely studied, others authors also observed the non-susceptibility of earthworms to Steinernematids (Capinera et al., 1982; Nguyen and Smart, 1991; Shapiro et al., 1993) and to the slug-parasitic nematode *Phasmarhabditis hermafrodita* Schneider (Nematoda: Rhabditidae) (Grewal and Grewal, 2003). However *S. feltiae* was highly virulent (100% mortality) against *S. littoralis* larvae before the nematode passed through the earthworm gut. The high virulence of this nematode against *S. littoralis* had been previously demonstrated by Abbas and Saleh (1998) and Glazer et al. (1991).

3.2. Phoretic transmission of *S. feltiae* by *E. fetida*

The percentage of earthworms transmitting nematodes through their gut ranged from 20 to 90% (Fig. 1A). The native *S. feltiae* strain was successfully transmitted by young and adults of *E. fetida* (83–92%), and significant differences were not observed between 24 and 48 h. The percentage of young earthworms transmitting the native strain was significantly higher than the commercial strain after 24 h ($\chi^2 = 55.123$, df = 1, $p < 0.01$), however no significant differences were observed after 48 h. No significantly differences in transmission within *S. feltiae* strains were observed in young and adults of *E. fetida* after 48 h. The percentage of earthworms transmitting nematodes from the commercial strain ranged from 17 to 78%, with statistical differences between young and adult observed at 24 h ($\chi^2 = 34.712$, df = 1, $p < 0.01$). The percentage of young earthworms transmitting significantly increased three times from 24 h to 48h ($\chi^2 = 29.980$, df = 1, $p < 0.01$).

The mean number of infective juvenile of *S. feltiae* observed in *E. fetida* casts is shown in Fig. 1B. The mean values observed ranged from 1 to 11 nematodes/earthworm. The highest value was observed for the adults of the commercial strain (11 IJs) after the first 24 h (cast I). Significant differences in the number of IJs between commercial and native strains were observed after 24 h in cast I of young ($Z = -2.232$, df = 1, $p = 0.026$), and adult ($Z = -2.104$, df = 1, $p = 0.035$) earthworms. However these differences were not observed in cast II. Statistical differences were not observed within young and adult earthworms to the same nematode strain for cast I and cast II. However, the number of IJs observed in cast I was significantly higher than cast II (native

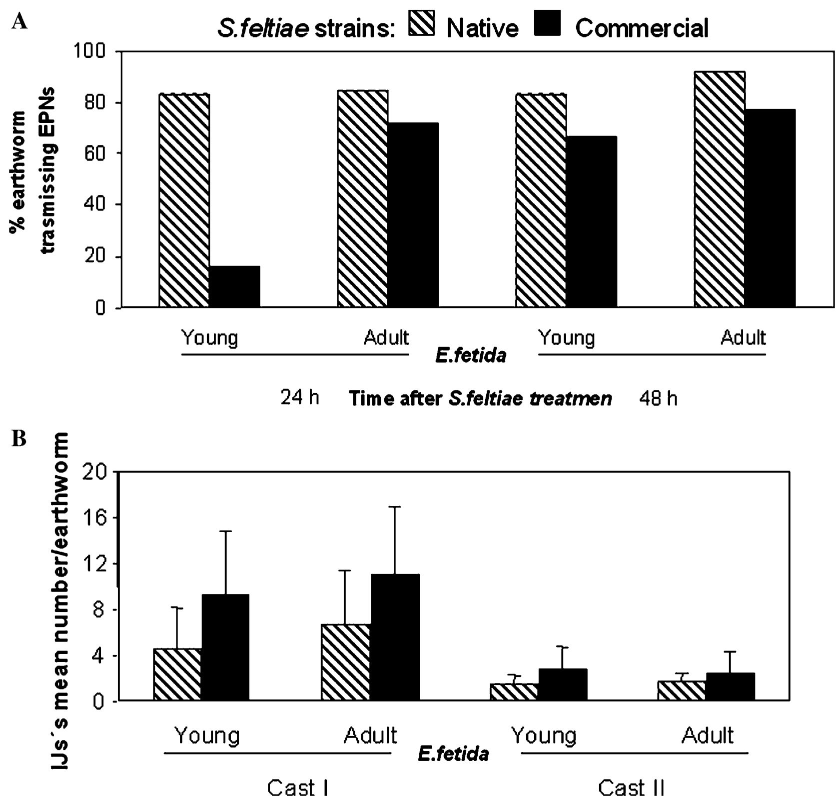

Fig. 1. (A) Percentage of young and adult earthworms transmissing EPN at 24 and 48 h post-exposure. (B) Average number of IJ's transmitted by the earthworm.

strain-young earthworm: $Z = -2.834$, df = 1, $p = 0.005$; native strain-adult earthworm: $Z = -2.680$, df = 1, $p = 0.007$; commercial strain-young earthworm: $Z = -2.404$, df = 1, $p = 0.016$; and commercial strain-adult earthworm: $Z = 4.851$, df = 1, $p < 0.001$). These results indicate that the transmission of IJs by young and adult earthworms was statistically higher in the first 24 h.

Phoretic associations between nematodes and earthworms were also described by Poinar (1978) for *E. fetida* and *Rhabditis maupasi* Seurat in Maupas (Rhabditida: Rhabditidae), and the occurrence of *Pellioditis pelio* (Schneider) Timm (= *Pelodera pellio* = *Rhabditis pellio*) (Rhabditida: Rhabditidae) in the excretory system of *Aporrectodea trapezoides* Dugés (Oligochaeta: Lumbricidae). The phoretic relationships between entomopathogenic nematodes and earthworms: *Steinernema carpocapsae* (Weiser) Wouts, Mráček, Gerdin, and Bedding (Rhabditida: Steinernematidae)—*A. trapezoides* and *Steinernema scapterisci* Nguyen and Smart (Rhabditida: Steinernematidae)—*Allolobophora caliginosa* Savigny (Oligochaeta: Lumbricidae) were also described (Nguyen and Smart, 1991; Shapiro et al., 1993). However this is the first time that a phoretic association between *S. feltiae* and *E. fetida* in laboratory trials is described.

3.3. Infective juvenile's activity after phoretic transmission

The mobility of *S. feltiae* infective juveniles was reduced after passage through the *E. fetida* gut (Table 1).

Table 1
Percentage of mobile IJs of *Steinernema feltiae* after passage through *Eisenia fetida* and IJs virulence against *Steinernema littoralis*

IJs thought earthworm gut	Native strain		Commercial strain	
	Young	Adult	Young	Adult
Cast I				
% mobile IJs	9.5	1.8	10.0	10.7
% *S. littoralis* mortality	0	0	0	15.4
Cast II				
% mobile IJs	0	0	0	15.0
% *S. littoralis* mortality	0	0	0	0

The percentage of mobile nematodes ranged from approximately 2–15%. The nematode virulence against *S. littoralis* larvae was 0% in all treatments except in cast I from commercial strain adults. These results demonstrated that passage through gut kills nematodes and drastically reduced its virulence. Soil microorganisms (bacteria, protozoa, algae, fungi, and nematodes) are digested in the earthworm gut as part of its diet (Atlanvinyte and Pociene, 1973; Bonkowski and Schaefer, 1997; Dash et al., 1980; Flack and Hartenstein, 1984; Hand et al., 1988; Moody et al., 1996; Piearce, 1978; Piearce and Phillips, 1980; Tiwari et al., 1990; Wolter and Scheu, 1999), although some of them survive through the gut passage (Doube et al., 1994; Moody et al., 1996; Stephens et al., 1995). Furthermore, the results obtained by Shapiro et al. (1993) related to the infectivity of

S. carpocapsae juveniles isolated in cast from *Lumbricus terrestris* L. (Oligochaeta: Lumbricidae) and *Aporrectodea trapezoides* on *G. mellonella* showed that this fact is possible. A subsequent survey (Shapiro et al., 1995) corroborated the possibility of a phoretic association within *S. carpocapsae* and *L. terrestris*, with infective juveniles viable after passage through the earthworm gut. The results of this study show a phoretic transmission of *S. feltiae* through *E. fetida* gut, with IJs being observed in the casts. Moreover poor mobility and slight virulence was observed, probably due to effect of digestive enzymes on nematode survival. On the other hand, it was observed that higher number of earthworms were able to transmit the IJs from the RIOJA strain than the commercial strain. However the number of native IJs observed in the casts was lower than the IJs from commercial strain, probably due to a differential intake of nematodes by *E. fetida*. The RIOJA strain was easily ingested by *E. fetida*, thus the 83–92% of the earthworm population showed JI in their casts, although they were very susceptible to *E. fetida* digestive enzymes. More studies are necessary to investigate this phoretic relationship.

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