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SHORT COMMUNICATION

A laboratory study on the activity of *Steinernema feltiae* (Rhabditida: Steinernematidae) Rioja strain against horticultural insect pests

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Abstract The potential of the entomopathogenic nematode *Steinernema feltiae* (Filipjev) (Rhabditida: Steinernematidae) Rioja strain was assessed under laboratory conditions. Last instar larvae of *Leptinotarsa decemlineata* Say (Coleptera: Chrysomelidae), *Spodoptera littoralis* Boisdouval (Lepidoptera: Noctuidae) and *Trichoplusia ni* Hübner (Lepidoptera: Noctuidae) were exposed to infective juveniles (IJs) under laboratory conditions. Larval mortality, days to larval death, infection cycle length, and reproductive potential were recorded. Efficacy was assessed performing dose–response experiments. The results indicate that control of *L. decemlineata* with *S. feltiae* Rioja strain is not economically profitable (LC_{50} = 99.61 IJs/cm^2), whereas results obtained for *T. ni* (LC_{50} = 0.27 IJs/cm^2) are promising. Due to the life cycle of this insect, the efficacy needs to be investigated in foliar application studies. The effects on *S. littoralis* (LC_{50} = 0.69 IJs/cm^2) was considered the most suitable for development of the Rioja strain as a biocontrol agent for soil application.

Keywords Entomopathogenic nematodes · *Leptinotarsa* · *Spodoptera* · *Steinernema* · *Trichoplusia* · Virulence

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Introduction

La Rioja (Northern Spain) is an agricultural region with high value crops such grapes, potatoes, cereals, vegetables, and fruits. Among other pest insects, *Leptinotarsa decemlineata* Say (Coleoptera: Chrysomelidae), *Trichoplusia ni* Hübner and *Spodoptera littoralis* Boisdouval (Lepidoptera: Noctuidae) are major pests in greenhouse and outdoor crops (Cabello et al. 1996; Pérez-Marín 2007). Some populations of these insect pest show resistance to several organophosphorus, synthetic pyrethroid, and *Bacillus thuringiensis* Berliner (Bacillales: Bacillaceae) insecticides (Shaaban et al. 1985; Ferre and van Rie 2002). The European and Mediterranean Plant Protection Organization (OEPP/EPPO) considers *S. littoralis* and *L. decemlineta* as A2 quarantine pests and recommends the development of novel control methods (OEPP/EPPO 2006). With an increasing trend for cropping under organic or integrated production practices (MAPA 2007) and the need to reduce the use of agrochemicals, it is important to develop alternative control methods against these pest insects.

Entomopathogenic nematodes (EPNs) in the Steinernematidae and Heterorhabditidae families are considered interesting candidates for use in biological control programs since they have a mutualistic symbiotic association with enteric bacteria that makes them strongly virulent to insects (Adams et al. 2006; Kaya et al. 2006). Several commercial products based on EPN are available in Europe (Kaya et al., 2006). However, since environmental conditions might affect survival, reproductive potential and virulence of the EPN strains, several countries are developing surveys to isolate those strains more adapted to local ecological conditions. This paper presents results from laboratory studies on a Mediterranean strain of *Steinernema feltiae* (Filipjev) (Rhabditida: Steinernematidae) (Rioja strain) and its activity against *L. decemlineata*, *T. ni* and *S. littoralis*.

Materials and methods

Nematodes and insect pests

Steinernema feltiae Rioja strain has been characterized in previous studies using *Galleria mellonella* Linnaeus (Lepidoptera: Pyralidae) as host (Campos-Herrera et al. 2006). This EPN strain was reared as described by Woodring and Kaya (1988) and infective juveniles (IJs) were stored at 10°C for 1–2 weeks before use. The IJ concentrations required for the bioassays were adjusted by volumetric dilutions in mQ water (Milli-Q Water System, Millipore S. A., Molsheim, France) following the method of Glazer and Lewis (2000).

The chrysomelid *L. decemlineata* was collected from potato field, *Solanum tuberosum* Linnaeus (Solanales: Solanaceae), from La Rioja (Northern Spain), and maintained under laboratory conditions on their host plant. A laboratory population of *S. littoralis* and *T. ni* were periodically infused with field-collected individuals from some crops from La Rioja and reared under laboratory conditions as described by Poitout and Bues (1974). Last instar larvae for all the assays/pest species were used in all experiments.

Dose–response bioassays: selection of suitable nematode–insect interaction

Assays were performed in 5-cm diameter Petri dishes containing 5 g of sterilized sand (1.6–0.16 mm particle size), as suggested Bedding et al. (1983). IJ suspensions were prepared to obtain 2, 12, 25 and 75 IJ/cm^2 each applied with 500 µl mQ water; controls received water only. Three Petri dishes with five larvae/dish were used for each concentration and control treatment. The experiment was repeated four times. Insect mortality and time to death of larvae was recorded daily during 7 days. Cadavers were rinsed in tap water to remove nematodes from their surface, individually placed in White traps (White 1927) and incubated until the new generation of IJs left the cadavers. The number of days until first IJ emergence was recorded to determine the length of the infection cycle and IJs produced after 10 days were counted to assess the reproductive potential (no. IJs/mg larva).

Statistical analysis

Insect mortality was corrected from the control treatment values using Abbott's formula (Abbott 1925) and the data obtained as percentage were arc sin transformed prior to the statistical analysis performed by SPSS 15.0 software (SPSS Inc., Chicago, IL, USA) for Windows XP. Data from the dose–response experiments were analyzed performing two-factor ANOVA analyses using as factors the insect species (3 groups) and EPN application dose (4 groups). Interaction between insect mortality, time to death of larvae, days from infection to IJs emergence, and reproductive potential (no. IJs/mg larva) was assessed. Analyses were repeated using the EPN application dose–insect species as variable, which contained all groups, performing the Duncan's paired test to assess significant differences ($P \leq 0.05$). Probit analysis from mortality data from dose–response experiments were carried out to calculate the LC_{50} and LC_{90}. A parallelism test was applied to regression lines, and LC_{50} and LC_{90} differences between insect were considered significant when 95% fiducial limits (FL) did not overlap.

Results

Larval mortality was affected by the nematode dose, the insect species and their interaction ($F = 9.33$, $df = 8, 90$, $P < 0.001$, $R^2 = 0.908$), showing a significant increase with the dose (Fig. 1A). Insect species differed in sensitivity to *S. feltiae* Rioja strain, indicating higher activity against the lepidopteran pests than to the chrysomelid *L. decemlineata.* Larval mortality was 70 ± 4.5% and 80.3 ± 9.5% at 2 IJs/cm^2 for *S. littoralis* and *T. ni*, respectively, increasing to over 95% at concentrations of 25 and 75 IJs/cm^2. Larval mortality for *L. decemlineata* was only 40 ± 10.3% at the highest IJ concentration of 75 IJs/cm^2. Significant differences in larval mortality were also observed among insect species at the same dosage (Fig. 1A).

The nematode dose–insect species interaction was not significant for the time to larval death, although the insect species significantly affected this variable ($F = 68.68$, $df = 2, 64$, $P < 0.001$) (Fig. 1B). *S. littoralis* and *T. ni* were very sensitive and died within a few days (3.5 ± 0.22 and 3.0 ± 0.10 days, respectively), with no significant differences among IJ concentrations. *L. decemlineata* was significantly more resistant than the lepidopteran hosts to nematode attack and took 5–6 days to die. When chrysomelid larvae were dissected under a stereoscopic microscope, it was observed that IJ inside the cadavers could not leave the cadaver after multiplication.

The nematode life cycle in both Lepidoptera species studied was only slightly affected by the insect species ($F = 8.94$, $df = 1, 48$, $P < 0.005$), with significant differences between insects species at the lowest concentration of 2 IJs/cm^2 (Fig. 1C). However, the reproductive potential was affected by the insect species ($F = 213.11$, $df = 1, 48$, $P < 0.001$) and nematode dose ($F = 4.31$, $df = 3, 48$, $P < 0.010$) without a significant conjunction effect (Fig. 1D). *S. feltiae* produced twice as many IJs in *T. ni* (173.8–204.8 IJs/mg larva) compared to *S. littoralis* (80.9–105.9 IJs/mg larva).

The results of the Probit analysis are presented in Table 1. The analysis model was accepted ($\chi^2 = 125.733$,

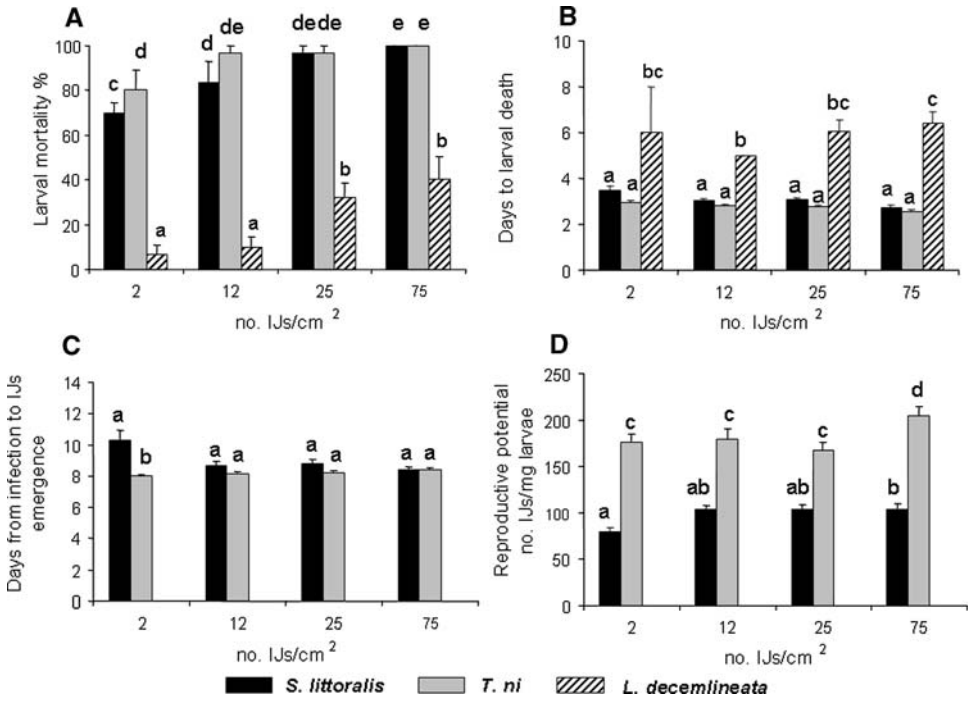

Fig. 1 Effect of increasing concentrations of *Steinernema feltiae* Rioja strain on *Spodoptera littoralis, Trichoplusia ni* and *Leptinotarsa decemlineata* larvae. **A** Mortality, **B** time to larval death, **C** time from infection to IJ emergence, **D** nematode reproductive potential. Data are expressed as means ± SEM. *Different letters* indicate significant differences after two-factor ANOVA and Duncan's paired test at $P < 0.05$

$df = 140$, $P = 0.800$) and the regression lines were considered parallel (Parallelism test, $\chi^2 = 0.000$, $df = 2$, $P = 1.000$) indicating that insect species response is qualitatively identical, but quantitatively different. The slope ± SEM estimated was 1.021 ± 0.128 for the three insect species, and the equations obtained were: *S. littoralis* Probit $(P_i) = 0.165 + 1.021$ ($\log_{10}$ concentration$_i$), *T. ni* Probit $(P_i) = 0.577 + 1.021$ ($\log_{10}$ concentration$_i$), *L. decemlineata* Probit $(P_i) = -2.040 + 1.021$ ($\log_{10}$ concentration$_i$). The lowest LC_{50} and LC_{90} values were recorded for *T. ni,* with 0.27 and 4.9 IJs/cm^2, respectively, followed by *S. littoralis* with values three times higher than for *T. ni* ($LC_{50} = 0.69$ and $LC_{90} = 12.4$ IJs/cm^2). For *L. decemlineata*, the highest LC_{50} and LC_{90} with 99.61 and 1,792.07 IJs/cm^2 were recorded, respectively. The results, given the lack of overlap of fiducial limits indicated that *S. feltiae* Rioja strain was significantly more virulent against the two lepidopteran pests than against *L. decemlineata.*

Table 1 Comparative virulence of the of *Steinernema feltiae* Rioja strain against *Spodoptera littoralis*, *Trichoplusia ni* and *Leptinotarsa decemlineata* larvae

Insect species	N[a]	LC_{50} (95% FL)[b]	LC_{90} (95% FL)[b]
S. littoralis	60	0.69 (0.27–1.32)	12.40 (7.50–149.57)
T. ni	60	0.27 (0.08–0.61)	4.90 (2.58–9.14)
L. decemlineata	60	99.61 (60.86–198.61)	1,792.07 (691.83–8199.52)

[a] N, number of individuals for each of the three insect species and applied concentration

[b] LC_{50}, LC_{90} and 95% FL values are expressed as number of *S. feltiae* Rioja strain IJs/cm^2

Discussion

Ecological studies of new EPN strains are strongly recommended to determine the range of their activity (Shapiro-Ilan et al. 2006), which depends on the species, strain characteristics, biotic and abiotic factors in the soil environment and the insect host species.

In this study, we showed that *S. feltiae* Rioja strain virulence against *L. decemlineata* was low with 40% larval mortality at 75 IJs/cm^2. Similar results were reported by Wright et al. (1987) with the Breton strain of *S. carpocapsae* with 30% at 158 IJs/cm^2. Therefore, we consider that the use of the Rioja strain is economically unprofitable for field applications ($LC_{50} = 99.6$ IJs/cm^2). Moreover, *S. feltiae* Rioja strain, as *Heterorhabditis marelatus* Liu and Berry (Rhabditida: Heterorhabditidae), could not complete their life cycle in *L. decemlineata* (Armer et al. 2004a). This incompatibility could be due to multiplex factors affecting the entry and establishment in the insect as well as the reaction of the insect immune system (Dowds and Peters 2002). Thurston et al. (1994) suggested that the low susceptibility of *L. decemlineata* might be due to the immune system activity, and Armer (2004b) suggested a poor nutritional value of insect host or a toxic factor expression in the hemolymph that could prevent the successful development of the nematode.

Steinernema feltiae Rioja strain was more active against Lepidoptera *T. ni* and *S. littoralis* larvae causing 97% mortality at 25 IJs/cm^2 to both species with reproductive potentials of 166.6 and 103.2 IJs/mg larvae, respectively. In dose–response experiments *S. feltiae* Rioja strain had a LC_{50} = 0.69 IJs/cm^2 (0.27–1.32, 95% FL) against *S. littoralis*. Differential sensitivity of *S. littoralis* to different species and strains of EPNs had been recorded earlier. A higher sensitivity to *S. carpocapsae* (All and Mexican strains), *H. bacteriophora* (HP88 strain) and *S. glaseri* than to *S. feltiae* was reported by Glazer et al. (1991) and Abdel-Razek (2006). However, *S. feltiae* Rioja strain was more virulent than *Steinernema abbasi* Elawad, Ahmad and Reid, *Steinernema riobrave* Cabanillas, Poinar and Raulston (Abbas and Saleh 1998), *Steinernema sangi* Phan, Nguyen and Moens and *Steinernema robustispiculum* Phan, Subbotin, Waeyenberge and Moens (Phan et al. 2005). *S. feltiae* Rioja strain virulence against *T. ni* (LC_{50} = 0.27 IJs/cm^2, 0.08–0.61, 95% FL) was higher than reported for *S. feltiae* UK and 27 strains with 4.7–9.5 IJs/cm^2, respectively, although quite different conditions were used (Belair et al. 2003). Although *T. ni* is more sensitive than *S. littoralis,* the habitat of *T. ni* is less suitable for use of EPNs because their larvae are foliar-feeding and foliar application decreases EPN activity due to desiccation, fluctuating temperature, and ultraviolet light (Grewal 2002). However, the use of protective formulation compounds and development of antidesiccants might improve their efficacy. Glazer et al. (1992) improved *S. carpocapsae* Mexican strain efficacy against *Earias insulana* Boisduval (Lepidoptera: Noctuidae), *Helicoverpa armigera* Hübner (Lepidoptera: Noctuidae) and *S. littoralis* in canopy applications to cotton plants. If nematode foliar applications were considered, the use of a calcium alginate gel formulation as carrier might improve the efficacy of *S. feltiae* (Navon et al. 2002). Moreover, *S. feltiae* Rioja strain has been reported as tolerant to several agrochemical products (Gutiérrez et al. 2008), so it might be considered a suitable candidate to develop as agent against pest under organic and integrated management of this species. Future studied will focus on the development of suitable ecological scenarios to enhance EPN activity, taking into account biotic and abiotic factors under laboratory and semi-natural condition such as greenhouse microcosms.

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