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Screening Spanish isolates of steinernematid nematodes for use as biological control agents through laboratory and greenhouse microcosm studies

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ABSTRACT

Entomopathogenic nematodes (EPNs) are one of the best non-chemical alternatives for insect pest control, with native EPN strains that are adapted to local conditions considered to be ideal candidates for regional biological control programs. Virulence screening of 17 native Mediterranean EPN strains was performed to select the most promising strain for regional insect pest control. *Steinernema feltiae* (Filipjev) (Rhabditida: Steinernematidae) Rioja strain produced 7%, 91% and 33% larval mortality for the insects *Agriotes sordidus* (Illiger) (Coleoptera: Elateridae), *Spodoptera littoralis* (Boisduval) (Lepidoptera: Noctuidae) and *Ceratitis capitata* (Wiedemann) (Diptera: Tephritidae), respectively, and was selected as the most promising strain. The *S. feltiae* Rioja strain–*S. littoralis* combination was considered the most suitable to develop the Rioja strain as a biocontrol agent for soil applications. The effect of soil texture on the virulence of the Rioja strain against *S. littoralis* was determined through dose–response experiments. The estimated LC_{90} to kill larvae in two days was 220, 753 and 4178 IJs/cm^2 for soils with a clay content of 5%, 14% and 24%, respectively, which indicates that heavy soils produced negative effects on the virulence of the Rioja strain. The nematode dose corresponding to the LC_{90} for soils with a 5% and 14% clay content reduced insect damage to *Capsicum annuum* Linnaeus (Solanales: Solanaceae) plants under greenhouse microcosm conditions. The results of this research suggest that an accurate characterization of new EPN strains to select the most suitable combination of insect, nematode and soil texture might provide valuable data to obtain successful biological control under different ecological scenarios in future field applications.

1. Introduction

Entomopathogenic nematodes (EPNs) from the Heterorhabditidae and Steinernematidae families are widely distributed in soils throughout the world and are considered to be one of the best non-chemical alternatives for insect pest control (Hominick, 2002; Kaya et al., 2006). However, applications of EPNs for biocontrol typically use non-native strains, despite the possibility that these introduced strains may not be well adapted to local environmental conditions and their survival, virulence and reproductive potential might be reduced, which could decrease their effectiveness as insect control agents. Furthermore, the introduction of exotic strains might have negative effects on non-target organisms, and might partially or completely displace native EPNs (Lynch and Thomas, 2000; Millar and Barbercheck, 2001), whereas the suitable management of native populations might avoid introductions of potentially invasive species (Grewal et al., 2002). Many countries have developed regulations that restrict the use of products based on non-native EPNs (Akhurst and Smith, 2002).Thus, the isolation of native strains by either sampling in various local habitats or in the habitat of a particular target host (Gaugler and Han, 2002) might be a very suitable approach for developing new biocontrol products and for avoiding future non-native nematode introductions. Moreover, evaluations of the virulence of recently isolated native EPN strains and studies of the effects produced by various biotic and abiotic soil characteristics on nematode efficacy might provide additional useful knowledge for biological control purposes (Lewis et al., 2006).

EPN strains have been isolated from various Spanish habitats and bioclimatic regions from natural hosts and soil samples, and some degree of genetic variability and different activities would be expected among these isolates (Gaugler et al., 1989). However, little information is available about their suitability as biological control agents (García del Pino, 2005). As result of our studies on EPN distribution, 21 steinernematid strains were recently isolated from La Rioja (northern Spain) (Campos-Herrera et al., 2006, 2007, 2008). In this region, economically important crops are cultured using organic or integrated production practices (MAPA, 2007). We selected the insect species *Agriotes sordidus* (Illiger) (Coleoptera: Elateridae), *Spodoptera littoralis* (Boisduval) (Lepidoptera: Noctuidae), and *Ceratitis capitata* (Wiedemann) (Diptera: Tephritidae) as targets of

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interest because they produce important economic damage to crops in Mediterranean countries, affect both horticultural and orchard crops in La Rioja (Pérez–Marín, 2007), and some populations are resistant to several organophosphorus, synthetic pyrethroid and Bt-insecticides (Ferre and van Rie, 2002; Shaaban et al., 1985). The European and Mediterranean Plant Protection Organization consider *S. littoralis* and *C. capitata* as A2 quarantine pests, and recommend the development of novel control methods (OEPP/EPPO, 2006). Virulence studies of EPN strains against *S. littoralis*, *C. capitata*, and *Agriotes* spp are rare (Abdel–Razek, 2006; Gazit et al., 2000; Phan et al., 2005; Schalk et al., 1993). Therefore, our aim was to select and characterize the activity of the most promising native EPN strain to be developed as a biological control agent against these insects. Our specific objectives were: (1) to evaluate 17 Riojan EPN isolates against *A. sordidus*, *S. littoralis* and *C. capitata* by assessing percent mortality, and time to kill, and to select the most active EPN strain against the three insect species; (2) to explore, under laboratory conditions, the effect of soil texture on nematode applications against selected nematode–insect combinations and to determine the lethal concentration (LC_{50-90}) to kill the insects in two or more days; (3) to test, under greenhouse soil microcosm conditions, the efficacy of previously calculated LC_{90}.

2. Materials and methods

2.1. Nematode strains, insects, soils and general procedures

The native strains 17, 23, 37, 38, 58A, 63, 66, 75, 91, 100, Rioja, BZ, BV, LV, and LFE of *Steinernema feltiae* (Filipjev) (Rhabditida: Steinernematidae) as well as strains 96 and 98 of *Steinernema carpocapsae* (Weiser) (Rhabditida: Steinernematidae) were isolated from soil samples and natural hosts, and biologically characterized using the greater wax moth larvae *Galleria mellonella* Linnaeus (Lepidoptera: Pyralidae) (Campos-Herrera et al., 2006, 2007, 2008). Steinernematids were reared as described by Woodring and Kaya (1988). 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).

Agriotes sordidus larvae were collected from *Solanum tuberosum* Linnaeus (Solanales: Solanaceae) fields in Vitoria (Northern Spain), and maintained under laboratory conditions on their host plant. Laboratory populations of *S. littoralis* and *C. capitata* were periodically infused with individuals from nature, and were reared under laboratory conditions as described by Poitout and Bues (1974) for Lepidoptera and Albajes and Santiago-Álvarez (1980) for Diptera. Pupae of *C. capitata* and last instar larvae of *S. littoralis* were used in all experiments.

Soils with different textures from La Higueruela, Fuensanta and Villena localities were selected (Table 1). Soils were taken at a depth of 2–20 cm, roughly mixed and transported to the laboratory. A subsample of 100 g of each soil was dried at room temperature, sieved through a 2 mm mesh screen, and processed for physical–chemical characterization by the Soil Analytical Service of the Centro de Ciencias Medioambientales, CSIC, Madrid (Spain). Soils were evaluated for sand, silt and clay content by the Bouyoucos (1962) method and their textures were assessed following the Soil Survey Staff (1994). Field capacity (FC) at pF 2.7 and wilting point (WP) at pF 4.2 were carried out by Richards' method (Duchaufour, 1975), and available water (FC–WP) and pH was evaluated from a 1:2.5 soil to mQ-water suspension. Soils were sterilized prior to laboratory and greenhouse bioassays, which were conducted by adding a suitable volume of water and nematode suspension to moisten the soil surface and obtain a value close to the field capacity.

2.2. Screening of EPN strains virulence. Selection of promising EPN strain

All native EPNs strains were virulent against *G. mellonella* (Campos-Herrera et al., 2006, 2007, 2008), and were tested against three insect pests, using a modified procedure from Bedding et al. (1983) with multi-plate recipients. The virulence bioassay against *S. littoralis* and *A. sordidus* was performed using 25-well plates (4 cm^2/well) (Sterilin Hounslow, Middlesex, UK) containing a thin layer (0.8 g/well) of sterile sand (1.6–0.16 mm particle size) dampened with 50 µl mQ-water. Nematode strains were applied in each well as 100 µl of mQ-water suspension and adjusted to 250 IJs/cm^2, and finally one last instar larva of *S. littoralis* or *A. sordidus* was added. Plates in which each well contained 150 µl of mQ-water and an insect larva served as controls. The bioassays were carried out in a growth chamber at 22 ± 1 °C, 55 ± 5% r.h., and L16:D8 photoperiod. Virulence of EPNs was assessed by recording daily insect mortality and time to death during a 12 day period. Cadavers were rinsed in tap water to remove nematodes from their surface, individually placed in White traps (White, 1927), and incubated under the above described conditions to test for the presence of EPNs inside the insects. Insects from which IJs did not emerge into White traps were dissected under a stereoscopic microscope to verify EPN occurrence inside the insect body.

Virulence against *C. capitata* was tested in 24-well plates (1.77 cm^2/well) (Costar culture clusters, Corning Costar Corporation, Cambridge, Massachusetts, USA). One 24 h old pupa was placed into each well along with 2 g of sterile sand, 275 µl mQ-water, and 100 µl of adjusted EPN suspension (250 IJs/cm^2, equivalent to 141 IJs/ cm^3). Plates with pupae of *C. capitata* covered with sand and 375 µl mQ-water were used as controls. The experiment was incubated for 12 days as described above, and pupal mortality was assessed by subtracting the number of emerged adults from the initial number of pupae (Gazit et al., 2000).

All bioassays were repeated three times (no. larvae *S. littoralis* and *A. sordidus* = 75; no. pupae *C. capitata* = 72), with their corresponding control treatments, and the most active EPN strain against the three insect species was selected for testing in dose–response bioassays.

2.3. Effect of soil texture on EPN efficacy: laboratory dose–response bioassays

Three agricultural soils with sandy loam (La Higueruela), loamy sand (Fuensanta) and sandy clay loam (Villena) textures were used to determine the effect of soil texture on the selected EPN strain–

Table 1
Characterization of the agricultural soil used in texture assays.

Locality	Texture type[a]	Percentage			Field capacity	Wilting point	Available water	pH
		Sand	Silt	Clay				
Fuensanta	Loamy sand	85	10	5	8.5	4.8	3.7	7.5
La Higueruela	Sandy loam	65	21	14	11.0	6.1	4.9	5.0
Villena	Sandy clay loam	54	22	24	12.5	7.8	4.7	7.2

[a] Soil Survey Staff (1994).