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Diversity, occurrence, and life characteristics of natural entomopathogenic nematode populations from La Rioja (Northern Spain) under different agricultural management and their relationships with soil factors

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Abstract

Natural entomopathogenic nematode (EPN) populations were surveyed in La Rioja (Northern Spain) during two consecutive years (2003–2005) to study their diversity, occurrence, and life characteristics under different agricultural management representing natural areas and perennial and annual crops from organic and conventional systems. Native EPN species and strains were identified using morphological and molecular characteristics. Virulence, infection cycle length and reproductive potential were assessed using *Galleria mellonella* larvae. The EPN occurrence was evaluated through abundance, recovery frequency, larval mortality percentage and EPN population density. EPNs were also related to selected soil physical and chemical variables as well as to some soil pollutants such as heavy metals and organochlorine pesticide residues. Only two steinernematids species were identified: *Steinernema feltiae* was observed throughout all seasons from natural and agricultural areas and *Steinernema carpocapsae* in summer and autumn of 2004 from perennial crops only. The virulence of native strains was lower than other previously isolated Spanish strain from natural areas or crop field edges. EPN abundance and recovery frequency indicated that habitat type might influence EPNs occurrence stronger than seasonality with the intensity of agricultural management inversely affecting their distribution. Moreover, clay, P_2O_5, Zn, Cu and hexachlorobenzene contents negatively correlating with EPN population density. We consider that agricultural management should be taken into account if EPNs are going to be used as biological control agents.

Keywords: *Steinernema carpocapsae*; *Steinernema feltiae*; Steinernematidae; Agricultural system; Distribution; Spain; Heavy metals; Organochlorine pesticide residues

1. Introduction

Entomopathogenic nematodes (EPNs) from Heterorhabditidae and Steinernematidae families are distributed worldwide (Hominick, 2002). They have a symbiotic association with enteric bacteria that makes them highly virulent to insect hosts (Boemare, 2002). EPNs are considered a promising non-chemical insect pest control alternatives because they are obligate parasite of a broad number of insect species, they actively locate their host, have high reproductive potential, can be massively reproduced *in vitro* and are harmless to vertebrates and plants (Kaya and Gaugler, 1993). Although the use of EPNs against insect pests is common, their efficacy can depend on soil characteristics, agricultural management, and competition with native EPNs (Glazer, 2002; Lawrence et al., 2006). In addition, temporal fluctuations of EPN

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populations indicate the importance of temperature and moisture changes due to seasonal changes (Půža and Mráček, 2007).

As part of the nematode community, EPNs could be affected by the same biotic and abiotic factors. Although the effects of agricultural management practices and soil pollutants have been extensively studied on other members of the nematode community (Freckman and Ettema, 1993; Korthals et al., 1996; Nagy et al., 2004), the impact on EPN diversity and occurrence is largely unknown (Hummel et al., 2002). To better understand the general biology and ecology of native EPNs, we carried out a survey in la Rioja (Northern Spain) to study: (1) the diversity, occurrence, and life characteristics of natural EPN population under different levels of disturbance associated with agricultural management in two consecutive years and (2) the relationships of natural EPN populations with soil factors (physical–chemical soil characteristics, heavy metals contents and organochlorine pesticide residues).

2. Material and methods

2.1. Samples collection and entomopathogenic nematodes isolation

An incomplete random block design (fixed factors: habitat type; random factor: year, season) was used to study the EPN native populations under annual and perennial crops with conventional and organic management and natural close-by areas in the area of Santo Domingo de la Calzada, Bañares and Leiva in the Oja-Tirón Valley, La Rioja (Northern Spain) (42°27′–42°30′N, 2°56′–3°02′W). The selected area (48 km^2) had the following characteristics: 400–500 mm mean annual rainfall, 11 °C mean annual temperature (Government of La Rioja, 2001a, b), 582–622 m asl altitude (GARMIN® GPS system), Calcaric Regosol (except one natural area defined as Calcic Chromic Luvisol) (Guerra and Monturiol, 1970; Soil Survey Staff, 1994) and potential vegetation series of *Cephalanthero longifoliae–Querceto faginiae* S., *Junipero thuriferae–Querceto rotundifoliae* S. and *Asparago acutifolii–Quercetum rotundifoliae* S. (Rivas-Martínez et al., 2001). Within the area, 18 sites corresponding to five habitat types with different land uses along a low to high disturbance gradient were established: natural areas adjacent to crop fields with scrub and grassland vegetation (NA, no. = 3), perennial vineyard with *Vitis vinifera* Linnaeus (Vitales: Vitaceae) and orchard with *Pyrus communis* Linnaeus (Rosales: Rosaceae) under both organic (OP, no. = 4) and conventional management (CP, no. = 5), and annual crops *Allium* sp. (Aspagales: Alliaceae) and *Capsicun* sp. (Solanales: Solanaceae) with *Triticum* sp. (Poales: Poaceae) as a rotation crop under both organic (OA, no. = 3) and conventional management (CA, no. = 3). Each crop system received the local practices for irrigation, fertilization, tillage and control of pathogens, weeds and insects without foreign EPN introductions. Natural EPN populations were evaluated from spring 2003 to winter 2005, with the exact sampling dates as follows: May 21–23, 2003; August 4–6, 2003; November 26–28, 2003; January 27–30, 2004; May 12–14, 2004; July 27–29, 2004; November 22–25, 2004 and February 7–9, 2005.

A sampling unit was a plot of 256 m^2 divided into 64 subplots of 4 m^2 (51 subplots in one conventional perennial crop) surveyed 8 times throughout 2 years. To avoid resampling the same area, soil samples were collected from randomly selected five subplots. Because La Rioja is characterized by high content of gravel and boulders, each soil sample (~2 kg) was a composite of five scoops taken at 2–20 cm soil depth with a hoe. Total of ~90 composite soil samples were recovered per each season. Soil was placed in polyethylene bags, gently mixed and closed to prevent moisture loss, transported to the laboratory at 12–15 °C and stored at the same temperature until processing.

Prior to the EPN isolation using the insect baiting technique (Bedding and Akhurst, 1975), soil samples were left at room temperature for 24 h. Three $8 \times 8 \times 2\,cm^3$ plastic boxes were filled with 100 cm^3 of each soil sample and adjusted to field capacity (FC) by adding mQ-water (Milli-Q Water System®, Millipore S.A., Molsheim, France). Ten last instars larvae of *Galleria mellonella* Linnaeus (Lepidoptera: Pyralidae) were placed on the surface of each soil box, boxes were covered with a lid, inverted and incubated in the darkness at 24 ± 1 °C and 55% relative humidity. Cadavers were recovered after 6 days, washed in tap water and individually placed on White traps to allow the emergence of infective juveniles (IJs) (White, 1927). When EPNs were not recovered from soil, the bioassay was repeated twice using another three 100 cm^3 soil subsamples and 10 fresh *G. mellonella* larvae to confirm the negative results (Hominick, 2002).

The relative abundance (no. positive sites/no. total sites) and recovery frequency (mean of no. positive samples in each site/no. total samples in each site) expressed as a percentage (Liu and Berry, 1995) were calculated for each habitat type. A soil sample was considered positive if any of the three soil subsamples lead to *G. mellonella* infection. Larval mortality (%) was derived from the means per three plastic boxes used in bioassay. Nematode population density was estimated by the Koppenhöfer et al. (1998) method using an equation

$$y = 10^{(-0.25+2.08 \log x)},$$

where x is the total number of nematode infected cadavers and y is the number of invading nematodes. If EPN had infected insects, 10 new *G. mellonella* larvae were placed on the soil samples, repeating this step until no more infected cadavers were recorded at least for two consecutive steps. In this case, population density value was obtained from mean value from the three plastic box used to each soil sample.