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Distribution of the entomopathogenic nematodes from La Rioja (Northern Spain)

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Abstract

Entomopathogenic nematodes (EPNs) distribution in natural areas and crop field edges in La Rioja (Northern Spain) has been studied taking into account environmental and physical–chemical soil factors. Five hundred soil samples from 100 sites of the most representative habitats were assayed for the presence of EPNs. The occurrence of EPNs statistically fitted to a negative binomial distribution, which pointed out that the natural distribution of these nematodes in La Rioja was in aggregates. There were no statistical differences ($p \leqslant 0.05$) in the abundance of EPNs to environmental and physical–chemical variables, although, there were statistical differences in the altitude, annual mean air temperature and rainfall, potential vegetation series and moisture percentage recovery frequency. Twenty-seven samples from 14 sites were positive for EPNs. From these samples, twenty isolates were identified to a species level and fifteen strains were selected: 11 *Steinernema feltiae*, two *S. carpocapsae* and two *S. kraussei* strains. *S. kraussei* was isolated from humid soils of cool and high altitude habitats and *S. carpocapsae* was found to occur in heavy soils of dry and temperate habitats. *S. feltiae* was the most common species with a wide range of altitude, temperature, rainfall, pH and soil moisture, although this species preferred sandy soils. The virulence of nematode strains were assessed using *G. mellonella* as insect host, recording the larval mortality percentage and the time to insect die, as well as the number of infective juveniles produced to evaluate the reproductive potential and the time tooks to leave the insect cadaver to determinate the infection cycle length. The ecological trends and biological results are discussed in relationship with their future use as biological control.

Keywords: *Steinernema carpocapsae*; *Steinernema feltiae*; *Steinernema kraussei*; Biology; Distribution; Ecology; Entomopathogenic nematodes; Spain; Steinernematidae

1. Introduction

Entomopathogenic nematodes (EPNs) from Heterorhabtidae and Steinernematidae families have a symbiotic association with enteric bacteria, forming the complexes: *Xenorhabdus*-Steinernematidae and *Photorhabdus*-Heterorhabditidae. These symbiotic associations result highly virulent to insects, killing them rapidly (Boemare, 2002; Boemare et al., 1997). Entomopathogenic nematodes are widely distributed in soils throughout the world (Adams et al., 2006; Hominick, 2002; Hominick et al., 1996), and are considered one of the best non-chemical alternatives to insect pest control due to their ability to actively locate insect-hosts as well as their high reproductive potential, capacity for mass production and the fact that they are harmless to vertebrates and plants (Burnell and Stock, 2000; Gaugler, 2002; Gaugler and Kaya, 1990; Kaya and Gaugler, 1993).

The application of non-native EPNs as biocontrol agents is used worldwide. Since environmental conditions influence survival, virulence and reproductive

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potential of the EPNs strains, the efficacy of biological control programs could be decreased. Many countries and researchers are concerned about release of non-native nematodes, because they may have negative effects on non-target organisms and may partially or completely displace endemic EPNs (Bathon, 1996; Ehlers and Hokkanen, 1996; Lynch and Thomas, 2000; Millar and Barbercheck, 2001). In order to increase the efficacy of biological control programs and hence reducing the environmental risks, several countries are developing surveys to isolate EPNs strains adapted to local ecological conditions, thereby restricting non-native nematode importations.

Recently, many surveys have been conducted in temperate areas of Europe: Austria (Hozzank et al., 2003) Belgium (Midituri et al., 1997), Bulgaria (Shishiniova et al., 1998, 2000), Czechoslovakia (Mráček et al., 1999a, 2005), Denmark (Nielsen and Philipsen, 2003), Germany (Sturhan and Ruess, 1999), Poland (Bednarek, 1998), Russia (Ivanova et al., 2000), Slovakia (Sturhan and Lisková, 1999), Switzerland (Steiner, 1996) and United Kingdon (Gwynn and Richardson, 1996). However there is little information available about on EPNs from Mediterranean countries: Egypt (Shamseldean and Abd-Elgawad, 1994) Greece (Menti et al., 1997), Italy (Tarasco and Triggiani, 1997; Triggiani and Tarasco, 2000), Palestinian Territories (Iraki et al., 2000), Spain (De Doucet and Gabarra, 1994; García del Pino, 2005; García del Pino and Palomo, 1996a,1997), and Turkey (Hazir et al., 2003; Kepenekci, 2002; Susurluk et al., 2001, 2003). Furthermore, studies of new strains about their biology, virulence and habitat preference can improve the efficacy of field applications, thus it can be selected virulent strains with environmental and physical–chemical soil requirements compatible with the site for the EPN application. In order to contribute to the knowledge about these organisms for regional biological control programs, the aim of this survey is to study the distribution, ecological requirements and virulence of EPNs from La Rioja (Northern Spain).

2. Material and methods

2.1. Samples collection and soil analysis

The survey was carried out from March to April of 2003. The most representative areas were selected taking into account environmental and physical–chemical soil factors. Bioclimatic region in La Rioja include 10 potential vegetation series (Fig. 1a): Oromediterranean region: 1, *Vaccinio myrtilli-Junipereto nanae* S.; Supramediterranean region: 2, *Ilici-Fageto* S.; 3, *Festuco heterophyllae-Querceto pyrenaicae* S.; 4, *Luzulo forsteri-Querceto pyrenaicae* S.; 5, *Junipero oxycedri-Querceto rotundifoliae* S.; 6, *Cephalanthero longifoliae-Querceto faginiae* S.; 7, *Junipero thuriferae-Querceto rotundifoliae* S.; 8, *Spiraeo obovaeta-Quercetum rotundifoliae* S.; and Mesomediterranean region: 9, *Asparago acutifolii-Quercetum rotundifoliae* S.; 10, *Rhamno lycioidis-Quercetum cocciferae* S. (Rivas-Martínez, 1987; Rivas-Martínez et al., 2001,2002). Soil type was evaluated following the studies of Guerra and Monturiol (1970) and the Soil Survey Staff (1994) (Fig. 1b) obtaining 11 soil types: 1, Haplic Calcisol; 2, Calcaric Cambisol; 3, Dystric Cambisol; 4, Calcaric Fluvisol; 5, Lithic Leptosol; 6, Rendzic Leptosol; 7, Calcic Chromic, Luvisol; 8, Calcaric Phaeozem; 9, Calcaric Regosol; 10, Dystric Regosol and 11, Humic Cambisol. Data of annual average air temperature and rainfall were recorded from the maps of Government of La Rioja (2001a,b) (Fig. 1c and d) and the altitude was assessed *in situ* using the GPS system GARMIN®. A total of 500 soil samples were colleted from 100 sites particularly those with a high incidence of insect populations (Hominick, 2002), such as natural areas (no. sites = 43), annual crop field edges of cereal and horticultural crops (no. = 38) and perennial crop field edges of fruit orchard and vineyards (no. = 19). Sampling habitats were classified as: natural grassland (NG), natural woodland (NW), natural woodland scrub (NWS), natural scrub grassland (NSG), natural scrub (NS), annual crop edge-scrub (AS), perennial crop edge-scrub (PS), annual crop edge-scrub-grassland (ASG), annual crop edge-woodland-scrub (AWS), perennial crop edge-scrub-grassland (PSG), annual crop edge-grassland (AG), and perennial crop edge-grassland (PG).

Five soil samples were collected from each site, at least two meters apart in transect sampling formation into an 18–20 m^2 plot. Each soil sample (approximately 1 kg) was taken at a depth of 2–20 cm (Campbell et al., 1998; Yoshida et al., 1998), placed in polyethylene bags to prevent water loss, transported to the laboratory under refrigerated conditions, and stored at 12–15 °C until EPN evaluation. Each sampling site was characterised by bioclimatic regions with the potential natural vegetation and soil types, habitat, altitude, annual average temperature and rainfall. For each soil sample, a 200 g portion was analysed for different edaphological variables. Soil moisture was calculated as: $\%H = (\text{fresh weigh} - \text{dry weigh}/\text{dry weigh}) \times 100$, where dry weigh was obtained after over dry soil at 70 °C for 5 days. The pH was measured from a 1:2.5 soil/mQ-water suspension. The sand, silt and clay contents were evaluated by Bouyoucos method (MAPA, 1975). In order to compare the soil moisture trough different soil texture types, the field capacity (FC) at pF 2.7 and wilting point (WP) at pF 4.2 were determined by Richards' method (Duchaufour, 1975) to calculate the available water in soil (FC-WP) in those positive samples for EPN presence. All analyses were performed by the Analytical Service of the Environmental Sciences Centre.

2.2. Isolation of entomopathogenic nematodes

Soil samples were roughly mixed and stored at room temperature for 24 h previous to the test for EPN occurrence, using the insect baiting technique (Bedding and