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Distribution of entomopathogenic nematodes in Southern Cameroon

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ABSTRACT

A first survey of entomopathogenic nematodes (EPN) was conducted in three agro-ecological zones of Southern Cameroon in 2007 and 2008. Entomopathogenic nematodes were recovered from 26 of 251 soil samples (10.4%). Three species, *Heterorhabditis baujardi*, *Steinernema* sp. A and *Steinernema* sp. B were found. The two steinernematids were considered unidentified species. Among the positive samples, 23 samples contained only *H. baujardi* (88.5%), two contained *Steinernema* sp. A co-occurring with *H. baujardi* (7.7%), and one sample contained *Steinernema* sp. B (3.9%). *H. baujardi* was frequent in forest and fruit crop (cocoa and oil palm plantations). *Steinernema* sp. A was found in a tree plantation of teak, *Steinernema* sp. B in a forest habitat. Nematodes were mostly present in acidic soils with pH ranging from 3.7 to 7.0. The highest EPN presence was recorded in sandy loam, sandy clay loam, sandy clay and clay soils. EPNs were not recovered in sand, loamy sand and clay loam soils. Using principal component analysis for elucidating the major variation patterns among sampling sites, four factors explaining for 73.64% of the overall variance were extracted. Factors were a combination of geographical (latitude, longitude, altitude), soil (pH, contents of sand, silt and clay, organic carbon, texture), and moisture (wilting point, field capacity) parameters as well as climatic parameters (mean annual rainfall, mean air temperature). Logistic regression and redundancy analyses (RDA) revealed that soil pH, longitude, available water and altitude were associated with presence and absence of EPN. Both logistic regression and RDA indicated that, increasing soil pH and longitude, associated with decreasing altitude, led to higher percentages of samples containing entomopathogenic nematodes.

1. Introduction

Soil-inhabiting entomopathogenic nematodes (EPN) are a widespread component of most terrestrial ecosystems and recorded from all continents with the exception of Antarctica (Hominick, 2002). They are of great interest because of their potential in regulating insect populations, particularly insect pests with a soil-dwelling phase (Kaya and Koppenhöfer, 1999). Species of the genera *Steinernema* and *Heterorhabditis* are the most common (Kaya and Stock, 1997).

To be effective as biological control agents of insect pests, EPN need to be adapted to the environmental conditions of the site of application (Bedding, 1990). The understanding of the parameters that affect the diversity and distribution of EPN species in the soil would help to select isolates that are best suited to a particular environment. The effects of factors such as geographical location, climatic conditions, habitat type and soil properties on the occurrence and distribution of EPN have been studied for several areas (*e.g.* Campos-Herrera et al., 2007; Mrácek et al., 2005; Mwaniki et al., 2008; Prasad et al., 2001b). However, no similar study has been conducted in Southern Cameroon.

The knowledge about the effect of a single variable on the incidence of soil-inhabiting EPN may yield significant and useful ecological insight. Yet, understanding the relationships between different variables affecting the ecology of soil-inhabiting EPN will increase the success of their use in biological control programs. When studying ecological relationships, the use of multivariate analyses in ecology can lead to more powerful and robust interpretations compared to those obtained when using univariate statistics (James and McCulloch, 1990; Quesada-Moraga et al., 2007). In view of this, the objective of the present study was to: (1) determine the distribution and species composition of soil dwelling EPN in Southern Cameroon, and to (2) apply two multivariate methods in a combined approach to evaluate the effects of a series of variables in combination on the distribution of EPN in natural and agricultural ecosystems.

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2. Material and methods

2.1. Site description

The survey was conducted in three of Cameroon's five agro-ecological zones: (1) The humid forest zone with a bimodal rain distribution. The zone extends over the major part of the south-Cameroonian plateau between 500 and 1000 m asl. It covers the Center, South and East regions. The climate is of "Guinean" type with an annual average temperature of 25 °C and annual average rainfall of 1500–2000 mm. The soils are ferralitic, acid and clayey (Fig. 1). (2) The humid forest zone with a monomodal rain distribution. This zone covers the littoral and south-west regions, as well as the coastal area of the South region. The average temperature varies between 22 and 29 °C and average annual rainfall ranges from 500 to 4000 mm. Soils include the volcanic slopes of Mount Cameroon, sediments of rock origin along the coast. These soils are more often fertile nitosols. (3) The Western Highlands covering the West and North-West regions with 3.1 million ha (6% of the national territory) and 2.6 million inhabitants (nearly 25% of the total population). Average temperature is around 19 °C; the annual average rainfall varies between 1500 and 2000 mm. The relief is diverse, with landscapes of medium mountains, characterized by a vegetation of savannah, plateau, depressed basins and plains crossed by gallery-forests.

2.2. Survey methodology, nematode isolation and soil sample characterization

In two surveys a total of 251 soil samples were collected in 244 locations during the rainy seasons. The first survey was carried out from September to November 2007; the second from March to June 2008. Seven transects, following agro-ecological gradients, were chosen based on the road accessibility and the presence of different habitats. Along each sampling transect, locations were chosen every 20 ± 3 km. One or more sites were sampled per location depending on the number of habitats found. At each site, four to five random soil sub-samples were collected at a depth of 0–20 cm, over an area of 2–4 m^2 and kept separately. Each sub-sample consisted of about 1 kg of soil. Between two sampling sites, hand trowels used to collect the soil were thoroughly rinsed. On the day of sampling, approximately 500 cm^3 soil of each sub-sample was placed in a clean container and incubated with 5–7 larvae of *Galleria mellonella* as bait (Bedding and Akhurst, 1975). The containers were inverted and kept in darkness at ambient temperature. Five days after incubation, the dead larvae were transferred to White traps and Koch's postulates were performed to test the virulence of the isolated EPN (Kaya and Stock, 1997). The color of the dead larvae of *G. mellonella* was evaluated. The nematodes emerging from cadavers were collected alive in deionized water with 0.1% formalin and stored at 15 °C. Isolate stocks were maintained on *G. mellonella* larvae, being re-inoculated on a regular basis (every 2 months).

For each positive site, the four to five random soil sub-samples were pooled and the composite soil sample was sieved (2-mm) and air-dried. Soil pH was measured in a water suspension (soil:water ratio of 1:2.5, w/v) (Thomas, 1996). Organic C was determined by spectrophotometric analysis after chromic acid digestion (Heanes, 1984). Soil texture was determined by the hydrometer method (Gee and Bauder, 1986). Soil moisture content at field capacity (FC) at pF 2.5 and wilting point (WP) at pF 4.2 were determined using the ceramic pressure plate extraction method on disturbed samples. To compare available soil moisture across soils of different textures, FC and WP were determined by Richards' method (Cassel and Nielsen, 1986).

Annual average temperature and rainfall data of the different localities were derived from reports and revised maps of the Directorate of Meteorology of the Ministry of Transports, from the ORSTOM data map (Ambassa-Kiki, 1988; INS, 2004) and from the Cameroon soil description book (Yerima and Van Ranst, 2005). Human population density data were obtained from the National Institute of Statistics at Yaoundé (Tables 1–3).

2.3. Nematode identification

Both molecular and morphologic/morphometric examinations were used for identification. However, DNA sequences analysis was considered as the first approach. Molecular characterization of the isolates was performed by analysis of the ITS rDNA sequences. DNA was extracted and amplified by PCR as described in Yilmaz et al. (2009). Purification, cloning, and sequence analysis were done as described by Khatri-Chhetri et al. (2010). The

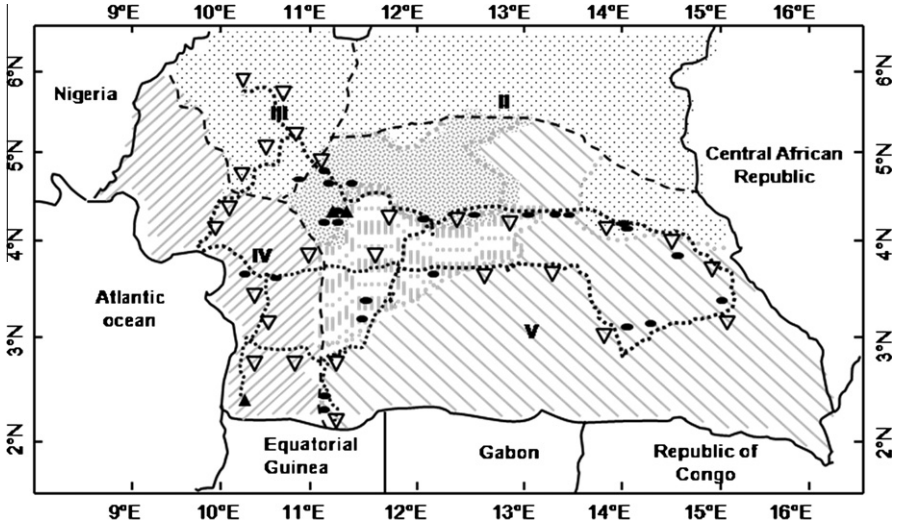

Fig. 1. Map of Southern Cameroon indicating the positive sampling sites. (II) Zone of mountainous guinea savannah. (III) Zone of western highlands. (IV) Humid forest zone with monomodal rainfall regime: Evergreen forest. (V) Humid forest zone with bimodal rainfall regime: Semi-deciduous forest, Savannah-forest transition zone, pre-forest sector, Mountain savannah, heterorhabditids, ▲ steinernematids; ▽ no entomopathogenic nematodes (this symbol often represents more than one sample), routes along which soil samples were collected.