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Isolation of entomopathogenic nematodes in the west region of Santa Catarina, Brazil

Isolamento de nematoides entomopatogênicos na região oeste de Santa Catarina, Brasil

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ABSTRACT: Entomopathogenic nematodes (EPNs) are potential candidate for integrated pest management programs. As little is known about the presence of these organisms in the state of Santa Catarina, it was aimed to perform soil sampling in the cities of Chapecó, Palmitos, Seara and Concordia for the isolation of EPNs. In total, 200 samples (100 g soil) were collected. In Chapecó, 40 samples from soil containing green manure (*Raphanus sativus*), five samples from native forest area and five samples from riparian forest were collected. In the city of Palmitos, 40 soil samples were obtained in the areas of soybean (*Glycine max*), corn (*Zea mays*), oats (*Avena strigosa*), and pasture (*Pennisetum purpureum*), and in each location 10 samples were taken. Sixty soil samples were collected in the city of Concordia, in a pasture area (*A. strigosa*). In Seara, the 50 soil samples were collected at a pasture consortium site between ryegrass (*Lolium multiflorum*) and black oats (*A. strigosa*). For the isolation, the collected soil samples were conditioned in 350 mL plastic containers and sent to the laboratory of the university. Later, four larvae of *Tenebrio molitor* of last instar were inserted, and the sets were maintained at the temperature of 25°C for seven days. After this period, the presence of dead larvae was verified, and the confirmation of the mortality by EPNs was evaluated using of White's trap. The positive samples for EPNs were obtained from the cities of Chapecó and Concordia, which corresponded to 2% of the total soil samples.

KEYWORDS: Nematoda; integrated pest management; biological control.

RESUMO: Os nematoides entomopatogênicos (NEPs) apresentam potencial para utilização em programas de manejo integrado de pragas. Como pouco se conhece sobre a presença desses organismos no estado de Santa Catarina, objetivou-se realizar amostragens de solo nas cidades de Chapecó, Palmitos, Seara e Concórdia para o isolamento de NEPs. No total foram coletadas 200 amostras (100 g solo). Em Chapecó, foram coletadas 40 amostras em solo contendo adubo verde (*Raphanus sativus*), cinco amostras de área de floresta nativa e cinco amostras de mata ciliar. Em Palmitos, foram obtidas dez amostras em cada área, totalizando 40. Foram elas: soja (*Glycine max*), milho (*Zea mays*), aveia (*Avena strigosa*) e pastagem (*Pennisetum purpureum*). Realizaram-se 60 amostras de solo na cidade de Concórdia, em área de pastagem (*A. strigosa*). Em Seara, as 50 amostras de solo foram retiradas em um local de consórcio de pastagem entre azevém (*Lolium multiflorum*) e aveia preta (*A. strigosa*). Para o isolamento, as amostras de solo coletadas foram acondicionadas em recipientes plásticos de 350 mL e alocadas no laboratório da universidade. Foram posteriormente inseridas quatro larvas de *Tenebrio molitor* de último instar, e mantiveram-se os conjuntos em temperatura de 25°C por sete dias. Após esse período, verificou-se a presença de larvas mortas, e a confirmação da mortalidade por NEP foi feita por meio de armadilha de White. As amostras positivas para NEPs foram obtidas da cidade de Chapecó (População 7, 18, 26) e Concórdia (População Concórdia), o que correspondeu a 2% do total de amostras de solo.

PALAVRAS-CHAVE: Nematoda; manejo integrado de pragas; controle biológico.

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The entomopathogenic nematodes (EPNs) are associated with symbiotic bacteria, and after penetration into the host insect the release of these symbionts causes mortality of the target within 72 hours after infection (FERRAZ et al., 2008; DILLMAN et al., 2012). EPNs have a potential as biological control agents since these organisms have a wide range of geographic areas, soil types and are adapted to several hosts. They do not cause damage to the environment and may also have synergistic effect with some phytosanitary products (GREWAL, 2012; LACEY, 2015; KAPRANAS et al., 2017).

In addition to the potential of these entomopathogens to control insects, studies have been developed to isolate these microorganisms from the soil and to perform pathogenicity tests on insects of agricultural importance (LU et al., 2016). Soil samples and isolation of EPNs in Brazil were made in the states of Minas Gerais (ACEVEDO et al., 2005; ANDALÓ et al., 2018), Rondônia (DOLINSKI; MOINO, 2006), Rio Grande do Sul (BARBOSA-NEGRISOLI et al., 2010; FOELKEL et al., 2017), São Paulo and Paraná (DE BRIDA et al., 2017).

In the agroecosystem, EPNs are affected by soil properties such as soil texture, moisture, temperature and organic matter, which might be drastically altered by agricultural management practices, such as crop rotation and cover crop rotation (JAFFUEL et al., 2016).

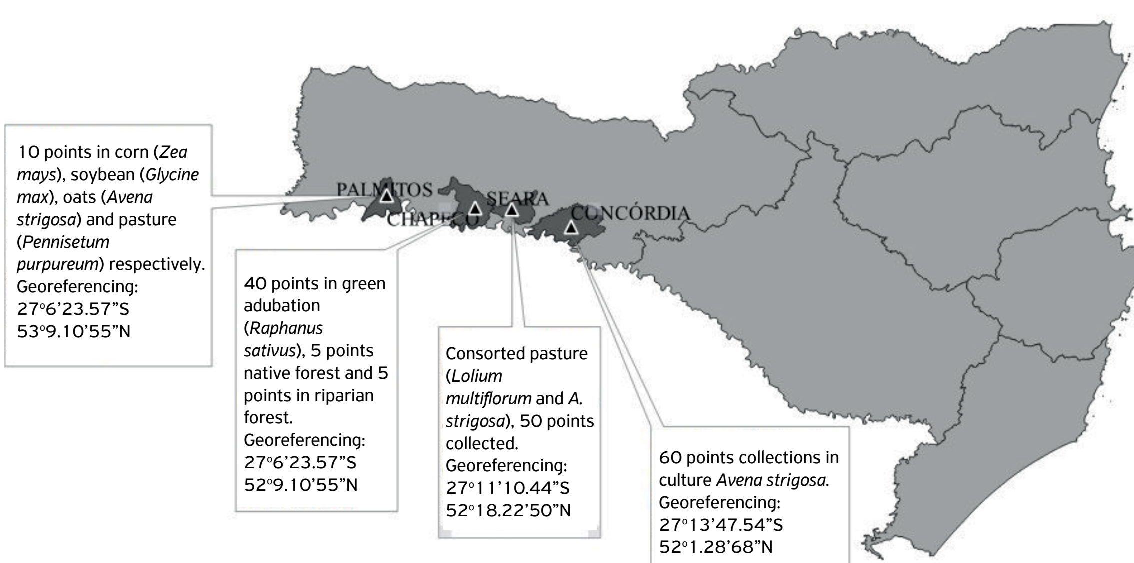
In the state of Minas Gerais, the natural populations of *Heterorhabditis amazonensis* were found in *cerrado* and gallery forest areas (ANDALÓ et al., 2018). In the state of Rio Grande do Sul, the occurrence of the species *Steinernema feltiae*, *Steinernema rarum* and *Steinernema riobrave* was reported for the first time in Brazil (BARBOSA-NEGRISOLI et al., 2010). Nematodes of the genera *Oscheius* are found in apple orchard (FOELKEL et al., 2017). EPNs also identified in agricultural areas of São Paulo and Paraná were *H. amazonensis*, *Metarhabditis rainai*, *Oscheios tipulae* and *S. rarum* (DE BRIDA et al., 2017).

In places where these microorganisms have not yet been explored, studies for sampling and isolation of EPNs are necessary (ACEVEDO et al., 2005). When EPNs are locally adapted, they provide effective control compared to exotic species (LU et al., 2016; RIVERA et al., 2016). Therefore, this study aimed to isolate EPNs in different agricultural areas of the west region of Santa Catarina, Brazil.

For the isolation of EPNs, surveys were conducted obtaining soil sampling from the cities of Chapecó, Palmitos, Seara and Concórdia between April and June 2017. According to Köppen's classification, the climate of the region is Cfa, with average annual temperature of 20°C and annual average rainfall of 1,830 mm (UHLMANN et al., 2012). The red clay latosol soil is predominant of western region of Santa Catarina state (POTTER et al., 2004).

Samples were collected from 10-cm depth from the soil surface, with the aid of a garden shovel, and the difference between each collection was at least 1 m apart. At each point of sampling, about 100 g of soil was collected, packed in plastic bags and transported to the laboratory in styrofoam box.

In Chapecó, 40 samples were collected in a revolved soil, containing previously *Raphanus sativus*, five samples in native forest area and five samples in riparian forest. In Palmitos, there were 10 samples in each area with annual crops: soybean (*Glycine max*), corn (*Zea mays*), oats (*Avena strigosa*) and pasture (*Pennisetum purpureum*), generating 40 samples. Sixty soil samples were collected in the city of Concórdia, in a pasture area (*A. strigosa*). In Seara, the 50 soil samples consisted of a pasture consortium between ryegrass (*Lolium multiflorum*) and black oats (*A. strigosa*). These collections totaled 200 soil samples (Fig. 1).



Source: QGIS DEVELOPMENT TEAM (2019).

Figure 1. Cities of Santa Catarina State where soil samples were collected for the isolation of entomopathogenic nematodes.

The insect-trap technique was used to obtain the EPNs. The soil samples were conditioned in plastic containers (9 × 12 cm) containing 100 g of soil and moistened with distilled water (when necessary). After that, four larvae of *Tenebrio molitor* L. (Coleoptera: Tenebrionidae) of last instar were added to each container, and all of them were closed with *voil* (BEDDING; AKHURST, 1975). The plastic containers were kept at the temperature of 25 ± 20°C in the Laboratory of Botany, Ecology and Entomology of Universidade Federal de Fronteira Sul.

Later, the dead larvae were transferred to a white trap at 25 ± 2°C for ten days. The infective juveniles (IJs) that left the *T. molitor* carcasses were collected daily with distilled water and stored at 18 ± 2°C. To purify and confirm the parasitism, Koch's postulates were applied by multiplications in larvae of *T. molitor*. The larvae of *T. molitor* were raised according to the methodology of POTRICH et al. (2007).

The cities that presented positive samples for EPNs were Chapecó and Concórdia. From the soil samples from Chapecó, three samples were positive to EPNs, obtaining three isolates — Population 7, Population 18 and Population 26 — from a total of 40 (7.5%) (Table 1). From the samples collected in the city of Concórdia, only one sample presented EPN, which corresponded to 1.6% of the total of 60 samples taken at the area (Table 1). The isolate captured at this site was called Population Concórdia.

In relation to the positive samples obtained, there are several favorable conditions that may have benefited the occurrence of EPNs in these areas, such as moisture, organic matter and associated cultures that favor the establishment of EPNs (LEWIS et al., 2015). Although these parameters were not directly compared in this study, soil samples positive for EPNs have clayey texture (POTTER et al., 2004).

The soils from the cities of Chapecó and Concórdia where EPNs were found are classified according to Brazilian Soil Classification System (SIBCs) as latosoil (EMBRAPA, 2013). These soils have as characteristics the silt content less than 20% and clay varying between 15 to 80%, and they are strongly to well drained (EMBRAPA, 2013). The clay texture did not interfere in the survival of EPNs, as reported by BARBERCHECK; KAYA (1991). Instead, each type of soil has a variety of unique characteristics that may have different effects on EPNs species (SHAPIRO et al., 2000).

In this study, it was observed that the cambisoils have medium or fine texture, while the argisoils can be sandy and have medium to clayey texture, and the nitosols are characterized by clayey to very clayey texture. However, these three soils have the common characteristic of good drainage. Anyway, organosoils come from predominantly organic material and are commonly associated with poorly drained environments, in which case the availability of oxygen may be difficult (EMBRAPA, 2013).

The soil texture can also influence the efficacy of the nematode. As the clay content increases, the nematode dispersion and the survival are influenced (SHAPIRO et al., 2000; KOPPENHOFER; FUZY, 2006). The moisture is one of the major factors affecting survival, virulence and persistence of IJs in the soil (LU et al., 2016), and different ranges of soil moisture affect the EPNs to find and infect a host (ACEVEDO; NÚÑEZ, 2003; SALAME; GLAZER, 2015).

In addition, optimum moisture levels will vary by nematode species and soil type, since excess moisture can cause oxygen deprivation and restrict movement. Soil characteristics must also be considered (SHAPIRO et al., 2006), as already mentioned in the soil texture. Studies generally report that lighter soils and soils with higher clay content restrict nematode movement and have reduced aeration potential, which may result in less nematode survival (GEORGIS; POINAR, 1983; MOLYNEUX; BEDDING, 1984). However, exceptions have been observed (SHAPIRO et al., 2000), and this study proved the occurrence of EPNs in soils with clayey texture (latosoil).

Table 1. Relationship of collection sites and positive samples for entomopathogenic nematodes.

Collection Location	City	Total number of collections	Number of positive samples for EPNs	Percentage of positive samples for EPNs (%)	Soil type
Green adubation (*Raphanus sativus*)	Chapecó	40	3	7,5	Latosoil
Native forestin	Chapecó	5	-	-	Organosoil
Riparian forest	Chapecó	5	-	-	Nitosoil
Corn (*Zea mays*)	Palmitos	10	-	-	Argisoil
Soybean (*Glycine max*)	Palmitos	10	-	-	Argisoil
Oats (*Avena strigosa*)	Palmitos	10	-	-	Argisoil
Pasture (*Pennisetum purpureum*)	Palmitos	10	-	-	Argisoil
Pasture (*Avena strigosa*)	Concórdia	60	1	1,6	Latosoil
Consorted pasture (*Lolium multiflorum* and *A. strigosa*)	Seara	50	-	-	Cambisoil
Total		200	4	2	

EPNs: entomopathogenic nematodes.

Several studies have demonstrated negative effects of intensive soil management (chemical fertilization, agrochemicals, monoculture, harrowing, among others) on EPNs (CAMPOS-HERRERA et al., 2012; CAMPOS-HERRERA et al., 2014; JAFFUEL et al., 2016). Though, the EPN populations isolated in the present study were found in agricultural areas. This fact can be justified by the adoption of smaller quantities of plant protection products, better vegetation cover on the soil and less soil rotation in the isolation areas when compared to soybean areas and corn.

The cultures to be implemented, as well as the history of the area (crop rotation), have an important effect on the abundance and activity of EPNs (JAFFUEL et al., 2016). In this study, the consortium of pastures favored greater root activity in the soil profile, such as nutrient recycling in the soil provided by *R. sativus*.

In a study of native EPN, 15.70% of the samples from the state of Rio Grande do Sul from the set of 121 soil samples collected contained EPNs. For the EPN positive samples, 7.69 to 18.18% were observed in forests, native pastures, fruit trees and corn, and between 21.42 and 25% were identified in soybean and tobacco. The species identified were *S. rarum*, *Heterorhabditis bacteriophora*, *H. amazonensis*, *S. feltiae*, *Steinernema glaseri* and *S. riobrave* (BARBOSA-NEGRISOLI et al., 2010).

In a verification survey of EPNs in the state of Minas Gerais, a total of 216 soil samplings was performed, from which three populations of EPNs were identified as *H. amazonensis* — two populations found in the *cerrado* area and one in the gallery forest (forest that forms corridor along rivers). Both areas are characterized by the presence of high-density vegetation cover, due to the characteristics associated with these biomes (ANDALÓ et al., 2018).

In a study investigating the presence of EPNs in the cities of Barretos, Botucatu, Garça, São Manuel, São Paulo, and Palotina, Paraná, samples were taken of agricultural soils with annual, fruit and forest crops, totaling 201 samples. From this total, 16 samples presented EPNs, which corresponded to 8%. Areas with positive samples were forest plantations (seven samples), annual crops (three samples) and orchards (six samples). The species identified were *H. amazonensis*, *M. rainai*, *O. tipulae* and *S. rarum*. EPNs samples were not found in plowed soil, native forest nor pasture areas. This result may also indicate the need for a higher number of samples collected at different soil depths (DE BRIDA et al., 2017). It demonstrates the difficulty in obtaining positive samples, even when they are collected in different environments.

This study did not present positive samples for EPNs in native forest nor in agricultural crops such as maize, which differs from the positive samples for EPNs found in other studies in Brazil (BARBOSA-NEGRISOLI et al., 2010; ANDALÓ et al., 2018; DE BRIDA et al., 2017). However, this study presented positive samples in pasture and soil areas with green manure, and it corroborates with BARBOSA-NEGRISOLI et al. (2010) and differs from the results presented by DE BRIDA et al. (2017).

The variation of sites with or without EPNs requires a great deal of effort mainly in sampling (sample area, number of samples, sample size), as well as the combination of extraction techniques of these soil microorganisms to avoid losses during the isolation of these entomopathogens (BARBOSA-NEGRISOLI et al., 2010; DE BRIDA et al., 2017). The isolation of the three populations in the west of Santa Catarina demonstrates the wide distribution of these organisms in Brazil, besides the potential use in pest control in the region, because they are adapted to the local conditions.

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