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Article

Meloidogyne Species Associated with Weeds in Rio Grande do Sul

*Espécies de **Meloidogyne** Associadas a Plantas Daninhas no Rio Grande do Sul*

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ABSTRACT - The frequency of species of root-knot nematodes (*Meloidogyne* spp.) was evaluated in weeds collected in different fallow farms in the State of Rio Grande do Sul, Brazil. In the samples where the nematode was found, the species of the root-knot nematode was identified by electrophoresis using the isozyme esterase. They were obtained from weeds belonging to 24 weed species from 13 different botanical families: Amaranthaceae, Asteraceae, Commelinaceae, Convovulaceae, Cyperaceae, Euphorbiaceae, Lamiaceae, Malvaceae, Oxalidaceae, Poaceae, Portulacaceae, Solanaceae, Verbenaceae. *Meloidogyne javanica* Est J3 (Rm: 1.0, 1.25, 1.40) was the most frequent species and occurred in 53.3% of the samples. *M. arenaria* with phenotype Est. A2 (Rm: 1.20, 1.30) was detected in 15.6% of the samples. *M. incognita* Est. I2 (Rm: 1.0, 1.1), *M. ethiopica* Est. E3 (Rm: 0.9, 1.15, 1.30), *M. enterolobii* Est. M2 (Rm: 0.7, 0.75, 0.9, 0.95) and *M. hapla* Est. H1 (Rm: 1.17) in 13.3%, 8.9%, 6.7% and 2.2% of the samples, respectively. Therefore, knowledge of the range of host plants to different species of the root-knot nematode can positively contribute to the adoption of management practices that allow the reduction of their populations in the soil.

Keywords: root-knot nematode; weed plants, isozyme esterase, characterization, hostability.

*RESUMO - A frequência de espécies do nematoide-das-galhas (**Meloidogyne** spp.) foi avaliada em plantas daninhas coletadas em diferentes lavouras em pousio do Estado do Rio Grande do Sul, Brasil. Nas amostras onde o nematoide foi encontrado, a identificação das espécies do nematoide-das-galhas foi feita por eletroforese, utilizando-se a isoenzima esterase. Elas foram obtidas de plantas daninhas pertencentes a 24 espécies de 13 famílias botânicas diferentes, sendo elas: Amaranthaceae, Asteraceae, Commelinaceae, Convovulaceae, Cyperaceae, Euphorbiaceae, Lamiaceae, Malvaceae, Oxalidaceae, Poaceae, Portulacaceae, Solanaceae and Verbenaceae. **Meloidogyne javanica** Est J3 (Rm: 1.0, 1.25, 1.40) foi a espécie mais frequente e ocorreu em 53,3% das amostras. **M. arenaria** com fenótipo Est. A2 (Rm: 1.20, 1.30) foi detectada em 15,6% das amostras. Também foram identificadas **M. incognita** Est. I2 (Rm: 1.0, 1.1), **M. ethiopica** Est. E3 (Rm: 0.9, 1.15, 1.30), **M. enterolobii** Est. M2 (Rm: 0.7, 0.75, 0.9, 0.95) and **M. hapla** Est. H1 (Rm: 1.17) in 13.3%, 8.9%, 6.7% and 2.2% of the samples, respectively. Portanto, o conhecimento da gama de plantas hospedeiras de diferentes espécies do nematoide-das-galhas pode contribuir de forma positiva para adoção de práticas de manejo que possibilitem a redução de suas populações no solo.*

Palavras-chave: nematoide-das-galhas, plantas infestantes, isoenzima esterase, caracterização, hospedabilidade.

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INTRODUCTION

The presence and interference of phytonematodes in agricultural areas is responsible for causing limitations to cropping systems, yield reduction and loss of quality of the resulting products. Among the major species, root-knot nematodes of the genus *Meloidogyne* are the most important group worldwide, mainly because of the wide variety of hosts, which may exceed 3,000 species of wild and cultivated plants (Hussey and Janssen, 2002; Moens and Perry, 2009). More than 100 species have been described for this genus. *M. arenaria*, *M. hapla*, *M. incognita* and *M. javanica* are the ones with the largest presence, accounting for about 95% of all infestations in agricultural areas (Brito et al., 2008).

In Brazil, species of *Meloidogyne* spp. are widely distributed in the different regions; moreover, they cause very severe damage and are difficult to control; therefore, annual and perennial crops, including soybeans, coffee, tomatoes, grapevines, sugarcane and cucumber, are subject to significant damage (Moens and Perry, 2009; Bellé et al., 2017a; Schmitt et al., 2018). In cropping areas, there is also a concomitant presence of different weed species. Such species may be parasitized by phytonematodes, and they have an important role in maintenance and multiplication of these specimens, especially during between harvests, where they are the predominant hosts in agricultural areas (Bellé et al., 2017b).

In addition, weeds multiply and ensure the maintenance of phytopathogenic organisms, including nematodes. Weeds pose a serious problem in agricultural production and can affect crops through competition for light, space, water and nutrients, as well as through release of allelopathic substances that interfere with plant growth and development (Santos and Cury, 2011; Gharabadiyan et al., 2012). In addition, the main weeds present in agricultural crops are resistant to herbicides, which increases their potential for damage and may reduce crop yield by more than 80% under high infestation conditions, depending on the occurring species (Carvalho et al., 2011). When weeds occur in areas affected by nematodes, their harmful potential is even higher, as they multiply these parasites and hinder the adoption of efficient control measures (Singh et al., 2010).

Species of the genus *Meloidogyne* are the group of phytonematodes with the highest frequency of infestation in weed roots (Ferraz et al., 1978). In this context, different weed species have been recognized as hosts of *Meloidogyne* spp. in different regions of the world and in Brazil (Mônaco et al., 2009; Kaspary et al., 2016; Groth et al., 2017). The problem of weeds as alternative hosts of these nematodes is particularly severe in subtropical and tropical environments, where weeds grow year-round (Kokalis-Burelle and Rosskopf, 2012). This fact leads to an increase of nematode populations in the soil, making it difficult to control them and aggravating the resulting damage in agricultural crops.

In view of the high polyphagic potential of phytonematode species of the genus *Meloidogyne*, knowledge of their wide range of host weeds is of paramount importance to select proper practices to manage these plant parasites. Thus, the aim of this research was to detect the presence of different species of nematodes of the genus *Meloidogyne* in different weeds in 15 towns of Rio Grande do Sul state, Brazil.

MATERIAL AND METHODS

Sampling took place in 15 towns of the state of Rio Grande do Sul, in fallow areas, from March to May 2018, in a total of 45 samples. Root samples were collected individually and stored in labeled plastic bags and taken to the Soil Biology Laboratory, Federal University of Santa Maria, for observations, analysis and recording of data. Weeds were identified and classified according to Lorenzi (2013).

Subsequently, 40 milky-white *Meloidogyne* sp. females were extracted from each weed root sample. Each female was then macerated in a capillary tube with 2-3 mL of extraction buffer for the enzyme esterase (sucrose buffer). Next, the protein extract from each sample underwent horizontal electrophoresis, according to the methodology described by Carneiro and Almeida (2001). The respective species (s) of *Meloidogyne* sp. were identified by esterase polymorphism (Est) in 7% polyacrylamide gel. As the standard of the enzyme esterase, the protein extract of

five females from a pure population of *M. javanica* (Est J3) was included in the gel (Carneiro and Almeida, 2001). Relative mobility (Rm) values of the polymorphic bands were calculated based on the first band of the standard *M. javanica*. The enzymatic phenotypes were tagged with a letter and a number, corresponding, respectively, to the name of each species and the number of bands with esterase activity (Esbenshade and Triantaphyllou, 1990). After that, the root samples were processed by the method of Coolen and D 'Herde (1972) by adding sodium hypochlorite to determine nematode population density per gram of root under an optical microscope.

RESULTS AND DISCUSSION

The study identified 24 weed species from 13 botanical families in the study areas infested with *Meloidogyne* sp. (Table 1). Weed species present in the different collection sites were only identified when they had root galls, namely: *Ageratum conyzoides* (goatweed), *Alternanthera tenella* (sanguinaria), *Amaranthus hybridus* (red amaranth), *Amaranthus spinosus* (spiny amaranth), *Amaranthus viridis* (slender amaranth), *Bidens pilosa* (hairy beggarticks), *Chenopodium album* (ÿþlamb's quarters), *Commelina benghalensis* (Benghal dayflower), *Cyperus rotundus* (purple nutsedge), *Echinochloa colonum* (jungle rice), *Eclipta alba* (false daisy), *Euphorbia heterophylla* (fireplant), *Galinsoga parviflora* (potato weed), *Ipomoea grandifolia* (morning glory), *Ipomoea nil* (morning glory), *Ipomoea purpurea* (morning glory), *Leonurus sibiricus* (honeyweed), *Oxalis corniculata* (creeping woodsorrel), *Portulaca oleracea* (common purslane), *Sida rhombifolia* (arrowleaf sida), *Solanum americanum* (American black nightshade), *Solanum pseudocapsicum* (Jerusalem cherry), *Solanum sisymbriifolium* (sticky nightshade) and *Verbena litoralis* (ÿþseashore vervain) (Table 1).

A total of 45 populations of *Meloidogyne* spp. were collected from the infected weed species, and six different esterase phenotypes were identified, corresponding to *M. javanica* Est. J3 (Rm: 1.0, 1.25, 1.40), *M. arenaria* Est. A2 (Rm: 1.20, 1.30), *M. incognita* Est. I2 (Rm: 1.0, 1.1), *M. ethiopica* Est. E3 (Rm: 0.9, 1.15, 1.30), *M. enterolobii* Est. M2 (Rm = 0.7, 0.75, 0.9, 0.95) and *M. hapla* Est. H1 (Rm: 1.17).

The most common root-knot nematode species was *M. javanica*, corresponding to 53.3%, identified in *A. tenella*, *A. spinosus*, *A. viridis*, *B. pilosa*, *C. benghalensis*, *C. rotundus*, *E. colonum*, *E. alba*, *E. heterophylla*, *I. nil*, *I. purpurea*, *O. corniculata*, *P. oleracea*, *S. rhombifolia*, *S. americanum*, *S. sisymbriifolium* and *V. litoralis*. For this nematode species, the number of second stage juveniles (J2) per gram of root ranged from 130 in *B. pilosa* to 1,250 in *C. benghalensis*, in the towns of Santo Ângelo and Tupaciretã, respectively. The species *M. arenaria* was detected in *A. viridis*, *C. album*, *C. benghalensis*, *E. heterophylla*, *I. grandifolia* and *P. oleracea*, corresponding to 15.6% of the analyzed samples, ranging from 120 to 620 J2 per gram of root. *M. incoginta* was found in 13.3% of the samples, in *I. grandifolia*, *A. conyzoides*, *E. colonum*, *S. americanum*, *G. parviflora* and *L. sibiricus*; the latter weed had the highest number of nematodes per gram of root: 560 specimens.

In this study, *M. ethiopica* (8.9%) was found in *I. purpurea*, *O. corniculata* and *S. rhombifolia*. *M. hapla* was detected in only one sample of *I. nil* (2.2%) in the town of Alpeste, where 318 J2 per gram of root were found. *M. enterolobii* was also found in 6.7% of the samples of *P. oleracea*, *S. rhombifolia* and *S. pseudocapsicum* collected in the town of Cachoeira do Sul, with 476, 350 and 675 specimens per gram of root, respectively (Table 1).

The presence of populations of *Meloidogyne* sp. in different weed species is indicative of the high level of polyphagia in this group of parasites, and corroborates previous studies that have reported their presence in several plant species (Hussey and Janssen, 2002; Moens and Perry, 2009). Thus, during weed management in agricultural areas, it is not feasible to prioritize the control of one weed species over another when it comes to reducing the phytonematode population. In this context, in the town of Cruz Alta, the species *M. javanica* was found parasitizing weeds of four different taxonomic families (Table 1): *O. corniculata O. corniculata* (Oxalidaceae), *E. heterophylla* (Euphorbiaceae), *S. americanum* (Solanaceae) and *C. rotundus* (Cyperaceae), indicating nonspecific parasite capacity and the need to control all weeds in areas with *Meloidogyne* (Table 1).

The high frequency of *M. javanica* infesting weeds, i.e., in more than 50% of the evaluated samples, shows its highest incidence in the different towns sampled. This high incidence has also been reported in a survey of 226 weeds, 49 of which were parasitized by *M. javanica* (Rich et al., 2009). Soybean is the main agricultural crop of the sampled municipalities, which may

explain the higher incidence of this species. *M. javanica*, since it is most often found parasitizing this crop (Kirsch et al., 2016). Thus, weeds that are often present and problematic in soybean, because of competition for environmental resources, are also frequently parasitized by these nematodes, as is the case with *Amaranthus* sp., *Ipomoea* sp., *Bidens pilosa*, *C. benghalensis* and *S. rhombifolia* (Table 1).

Table 1 - *Meloidogyne* populations, number of Meloidogyne per gram of root (NMGR) and esterase (EST) phenotype diagnosed from root samples of different weeds collected in 15 towns of Rio Grande do Sul, Brazil

Town	Scientific name	NMGR	EST	Meloidogyne species
Alpestre	*Leonurus sibiricus*	560	I2	*M. incognita*
	Ipomoea nil	318	H1	*M. hapla*
	Ageratum conyzoides	210	I2	*M. incognita*
	Echinochloa colonum	138	I2	*M. incognita*
Cachoeira do Sul	*Solanum pseudocapsicum*	675	M2	*M. enterolobii*
	Sida rhombifolia	350	M2	*M. enterolobii*
	Portulaca oleracea	476	M2	*M. enterolobii*
Caiçara	*Amaranthus spinosus*	674	J3	*M. javanica*
	Ipomoea nil	275	J3	*M. javanica*
Cruz Alta	*Oxalis corniculata*	502	J3	*M. javanica*
	Euphorbia heterophylla	210	J3	*M. javanica*
	Solanum americanum	980	J3	*M. javanica*
	Cyperus rotundus	242	J3	*M. javanica*
Frederico Westphalen	*Galinsoga parviflora*	518	I2	*M. incognita*
	Commelina benghalensis	210	J3	*M. javanica*
	Oxalis corniculata	340	E3	*M. ethiopica*
Ijuí	*Ipomoea grandifolia*	196	I2	*M. incoginta*
	Solanum americanum	374	I2	*M. incognita*
Novo Barreiro	*Ipomoea grandifolia*	620	A2	*M. arenaria*
	Portulaca oleracea	120	A2	*M. arenaria*
	Amaranthus viridis	540	A2	*M. arenaria*
	Chenopodium album	380	A2	*M. arenaria*
Palmeira das Missões	*Amaranthus viridis*	652	J3	*M. javanica*
	Amaranthus hybridus	450	J3	*M. javanica*
	Verbena litoralis	338	J3	*M. javanica*
Panambi	*Eclipta alba*	420	J3	*M. javanica*
	Solanum sisymbriifolium	204	J3	*M. javanica*
Santa Rosa	*Portulaca oleracea*	682	J3	*M. javanica*
	Bidens pilosa	130	J3	*M. javanica*
	Echinochloa colonum	430	J3	*M. javanica*
Santo Ângelo	*Ipomoea nil*	540	J3	*M. javanica*
	Bidens pilosa	130	J3	*M. javanica*
Sarandi	*Euphorbia heterophylla*	450	A2	*M. arenaria*
	Portulaca oleracea	250	A2	*M. arenaria*
	Commelina benghalensis	206	A2	*M. arenaria*
Seberi	*Alternanthera tenella*	98	J3	*M. javanica*
	Commelina benghalensis	375	J3	*M. javanica*
	Sida rhombifolia	690	J3	*M. javanica*
Tupanciretã	*Commelina benghalensis*	1250	J3	*M. javanica*
	Ipomoea purpurea	565	J3	*M. javanica*
	Sida rhombifolia	670	J3	*M. javanica*
	Amaranthus hybridus	850	J3	*M. javanica*
Vicente Dutra	*Sida rhombifolia*	502	E3	*M. ethiopica*
	Ipomoea purpurea	374	E3	*M. ethiopica*
	Oxalis corniculata	632	E3	*M. ethiopica*

Nematodes of the species *M. arenaria* were detected in 15.6% of the weed samples, especially in Sarandi and Novo Barreiro, which are neighboring towns; this is indicative that they are distributed in the region. The species *A. viridis and E. heterophylla* showed the largest number of *M. areanaria* specimens: 540 and 450 specimens per gram of root, respectively (Table 1). Corroborating the findings of this research, this species of nematodes has already been reported parasitizing several weeds, e.g., *P. oleracea*, *Aeschynomene americana* and *A. viridis* (Kokalis-Burelle and Rosskopf, 2012; Kaspary et al., 2016).

The species *M. incoginta* was found in 13.3% of the samples, in *I. grandifolia*, *A. conyzoides*, *E. colonum*, *G. parviflora*, *L. sibiricus* and *S. americanum* (Table 1). This result is different from the usually high proliferation of this species in weeds as well as its ability to parasitize more than 25 native weeds from Rio Grande do Sul (Bellé et al., 2017b). However, this low occurrence may result from the absence of weeds preferentially infested by *M. incoginta*, as a result of good management of invasive species. There was also a weak presence of other nematode species: 8.9%, 2.2% and 6.7% for *M. ethiopica*, *M. hapla* and *M. enterolobii*, respectively. These species were the least frequent in the cropping systems adopted in the sampled areas.

Infestation of common weeds by root-knot nematodes in various crops increases the harmful potential of such weeds. Other factors include the ability to compete for environmental resources, release allelochemicals and reduce the quality of the end product and the potential of these parasites to multiply. Therefore, it is difficult to adopt efficient control measures (Singh et al., 2010). For this reason, it is essential to adopt management practices that allow proper control of these weeds in order to reduce the population of this pathogen (Singh et al., 2013; Bellé et al., 2017a). Thus, integrated management strategies should be adopted for management of *Meloidogyne* sp., considering weeds as a source of inoculum for these parasites. Thus, chemical or cultural weed control, crop choice decisionsÿþ and intercropping of cultivated species in infested areas are of paramount importance for successful management of root-knot nematodes.

Therefore, weed control is a very important practice for management of nematodes, during the crop cycle and between harvests, in order to control the host and prevent the reproduction of this parasite, thus reducing damage caused to commercial crops (Singh et al., 2014; Bellé et al., 2017b). Furthermore, broader knowledge of the range of alternative hosts of *Meloidogyne* sp., especially in weeds, can aid the adoption of more effective pathogen management measures and enhances crop results.

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