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Prospecting for Nematodes Associated With Different *Prunus* Rootstocks in Brazil

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Abstract

The aim of the present study was to carry out a search for phytonematodes to verify the abundance and frequency of nematodes present in a peach orchard at eight years of age. Soil and root samples from nine peach rootstocks were collected in an experimental area in the district of Capão do Leão, in the State of Rio Grande do Sul, Brazil. After extraction, the samples were evaluated under an optical microscope. Nematodes of the following genera were identified in the samples: *Meloidogyne* spp., *Pratylenchus* spp., *Helicotylenchus* spp., *Mesocriconema* spp., *Trichodorus* spp., *Longydorus* spp., *Tylenchorchynchus* spp., *Hemiciclyophora* spp., *Xiphinema* spp., *Tylenchulus* spp., *Dorylaimus* spp., *Rotylenchulus* spp. and *Mononchus* spp., all associated with the roots or soil rhizosphere of 'Aldrighi', 'Capdeboscq', 'Flordaguard', 'Nemaguard', 'Okinawa', 'Tusukuba', 'Umezeiro', 'Viamão' and 'Industry' rootstocks. The most frequent phytoparasitic nematodes were *Meloidogyne* spp., and *Mesocriconema* spp., which, under high populations or under inadequate management of the cultivated area, represent a risk to peach cultivation. *Xiphinema* and *Pratylenchus* are two other phytonematodes that can cause damage to peach trees and were identified in greater abundance in the 'Industry', 'Viamão' and 'Nemaguard' rootstocks. Under the conditions of the present research the 'Okinawa' and 'Umezeiro' rootstocks proved to be unfavourable hosts, especially for *Meloidogyne* spp. and *Mesocriconema* spp. The 'Viamão', 'Capdeboscq', 'Aldrighi' and particularly the 'Industry' rootstcks should not be recommended for use in areas with an incidence of the nematodes *Meloidogyne* spp. or *Mesocriconema* spp.

Keywords: horizontal distribution, *Meloidogyne* spp., *Mesocriconema* spp.

1. Introduction

The peach [*Prunus persica* (L.) Batsch] is one of the most-produced temperate fruits in the world, being highly appreciated both for *in natura* consumption, and in the canning industry, it is processed for pulp, jam, juice and nectar (Usda, 2018; Zhao, 2017). In 2015, Brazil ranked 13th in the world in terms of peach and nectarine production, with 218 thousand tons (FAO, 2018).

The State of Rio Grande do Sul stands out as the largest producer in Brazil, with approximately 61% of the planted area (IBGE, 2018), although it is still the state with the lowest average productivity, around 10 ton ha^{-1} (Mayer et al., 2017).

The use of rootstocks with no genetic identity and susceptible to phytonematodes is one of the factors that has contributed to the low average productivity of peach orchards in Rio Grande do Sul (Mayer et al., 2017; Claverie et al., 2011). The occurrence of phytonematodes in stone-fruit trees has been associated with the decline of the plants in different producing regions around the world (Walters et al., 2008; Pinochet et al., 1996). In Brazil, there is a wide range of *Prunus* spp. rootstock introduced from other countries, each with a certain set of advantages and limitations for adaptation to different geographic regions (Almeida et al., 2015). Some cultivars are referred to as resistant to root-knot nematodes (*Meloidogyne* spp.), such as Nemared (Ramming & Tanner, 1983), Flordaguard (Sherman et al., 1991), Guardian (Nyczepir et al., 1999), Okinawa (Paula et al., 2011), Umezeiro (*Prunus mume*) (Lecouls et al., 1997; Mayer et al., 2017) and MP-29 (Backman et al., 2012). Other genotypes with characteristics of interest are being investigated in Brazil, such as rootstocks of the Okinawa-roxo, Tsukuba 1, Tsukuba 2 and Tsukuba 3 cultivars (Souza et al., 2016; Souza et al., 2017a), as well as interspecific hybrids introduced and used successfully in other countries, and other species of *Prunus* spp.

(Bianchi et al., 2004). However, few field studies have evaluated the potential of each of these genotypes under the conditions found in Brazil.

Given the importance of peach cultivation in Rio Grande do Sul, it is necessary to study the effective responses of the different rootstocks on population dynamics and the occurrence of phytoparasitic nematode species in stone fruits, not only under controlled conditions, but also under the conditions found in the field (Brida et al., 2017; Souza et al., 2017b).

Surveys of peach orchards in Rio Grande do Sul recorded a higher frequency of the species *Meloidogyne javanica* (Treub) Chitwood and *M. incognita* (Kofoid & White) Chitwood in the root system of the peach trees (Campos et al., 2002). A similar occurrence was recorded by Ferreira et al. (2007) in peach orchards in the State of São Paulo, Brazil.

In addition to the peach trees (*Prunus persica*), different species of the genus *Meloidogyne* spp. have also been regularly associated with damage in the acerola cherry (*Malpighia emarginata* DC), guava (*Psidium guajava* L.), fig (*Ficus carica* L.), mango (*Mangifera indica* L.), kiwi (*Actinidia chinensis* Planch.) and grape (*Vitis vinifera* L.) (Junqueira et al., 1999; Paula et al., 2011; Martins et al., 2013; Cavichioli et al., 2014; Souza et al., 2015a; Castro et al., 2016).

The lesion nematodes, *Pratylenchus penetrans* and *Pratylenchus vulnus*, are species that can also damage peach production, causing degeneration of the root system, predisposing the plant to infections caused by other phytopathogenic microorganisms, and are the primary causal agents of replant diseases in the peach, a problem characterised by the atrophy and yellowing of the plants, usually accompanied by root necrosis (Pinochet et al., 1993; Gomes & Campos, 2003).

Evaluating the association of Peach Tree Short Life Syndrome with soil fertility in the orchard, Mayer et al. (2015) found that the occurrence of early death in the peach was not related to the chemical attributes of soil fertility. In turn, Carneiro et al. (1993) and Campos et al. (2002) did not show any correlation between the occurrence of short life syndrome and the age of the plants, the location of the plants in the orchard, individual plants or groups of plants, not even with the scion. On the other hand, the ring nematode *Mesocriconema xenoplax* (Raski) Loof, has been associated with Peach Tree Short Life Syndrome (PTSL), whose symptoms are usually identified at the end of the dormancy period, and are characterised by a reduction or paralysation of growth and a reduction or lack of budding and flowering, leading to the death of the plants (Reddy et al., 2014). Parasitism by *M. xenoplax* in the peach tree causes darkening of the root system, followed by destruction of the tissue and atrophy of the roots (Kuhn et al., 2015), resulting in production problems, especially in Rio Grande do Sul, as it occurs almost everywhere stone fruits are cultivated (Mayer & Ueno, 2012; Carneiro et al., 1993).

In Brazil, in addition to little being known about the population dynamics of phytonematodes associated with the cultivation of stone fruit, there is no registered nematicide for these crops (Agrofit, 2018), so genetic resistance and crop rotation are the most sustainable, effective and economical practices to suppress or reduce the damage caused by these root parasites (Walters et al., 2008; Pinochet et al., 1996; Salesses et al., 1995). Adopting such practices are important during the initial establishment and productive life of the orchards, particularly in areas with a history of parasitic nematodes.

Faced with this problem, the aim of the present study was to carry out a search for phytonematodes to verify the abundance and frequency of nematodes present in the rhizosphere and roots of nine peach rootstocks in an orchard at eight years of age.

2. Method

Prospecting for nematodes was carried out in an orchard located at the Palma Agricultural Centre of the Federal University of Pelotas-UFPel, in the district of Capão do Leão, Rio Grande do Sul, Brazil (31º52′00″ S, 52º21′24″ W, altitude 40 m).

The soil in the orchard is moderately deep, with an average texture in the A horizon and a clayey texture in the B horizon, and is classified as a typic dystrophic Red-Yellow Argisol (Souza et al., 2017c). The mean cold accumulation in the region, below 7.2 °C, is around 400 hours, the mean annual rainfall is 1,367 mm, and the mean annual minimum and maximum temperatures are 13.8 °C and 22.9 °C respectively (EAP, 2018).

The nematological survey was carried out on two strips of land, one adjacent to the other, forming contiguous lines. The first (1) strip corresponds to an orchard of the Chimarrita and Maciel cultivars grafted onto eight rootstocks of *Prunus* spp. obtained from seeds of cultivars of known origin. The second (2) strip corresponds to an orchard of the Maciel cultivar grafted onto rootstocks obtained from stones from the canning industry, and therefore of no known origin, identified only as 'Industry'. Both orchards are eight years of age.

A total of 92 soil and root samples were collected in Strip 1, containing *Prunus persica* rootstocks identified as 'Viamão', 'Capdeboscq', 'Aldrighi', 'Tsukuba', 'Okinawa', 'Nemaguard' and 'Flordaguard', as well as 'Umezeiro' (*Prunus mume*), all grafted with the Chimarrita and Maciel cultivars. In Strip 2, the rootstock identified as 'Industry' was grafted with the Maciel cultivar, from which 50 soil and root samples were collected. In both strips, planting was at a spacing of 5.0 × 1.5 m.

Soil and root collections were made in the canopy projection area, at a depth of approximately 30 cm, discarding the top five centimetres of soil. The samples were packed in plastic bags, identified and sent to the Laboratory of Plant Molecular Physiology of the Federal University of Pelotas, Capão do Leão, Rio Grande do Sul.

To extract the soil nematodes, 250 mL were processed following the methodology proposed by Jenkins, (1964). For analysis of the roots, samples weighing 10 g were processed as per the extraction method described by Hussey and Barker (1973). The extracted nematodes were collected in plastic containers. The nematodes were counted using a Peters slide under an optical microscope. Temporary slides were prepared to identify the nematodes at the genus level, using the Nickle (1991) and Tihohod (1997) keys for this step.

After identifying the nematode populations extracted from the soil and root samples, the mean abundance of nematodes in the soil (Sa) and roots (Ra) was calculated, determined from the average number of taxa in the collected samples. The relative abundance (Ra%) in the soil and roots was also calculated as per Norton (1978), cited by Silva et al. (2008), with the formula Ra% = (A × 100)/N, where *A* is the number of individuals of a given taxon in the sample and *N* corresponds to the total number of phytoparasitic nematodes in the sample; the relative frequency (Rf%) was calculated with the formula Rf% = (na × 100)/Na, where *na* represents the number of samples in which a given nematode taxon occurred and *Na* is the total number of samples collected in a given rootstock.

3. Results and Discussion

Thirteen nematode genera were identified in the soil and root samples: *Meloidogyne* spp., *Pratylenchus* spp., *Helicotylenchus* spp., *Mesocriconema* spp., *Trichodorus* spp., *Longydorus* spp., *Tylenchorchynchus* spp., *Hemiciclyophora* spp., *Xiphinema* spp., *Tylenchulus* spp., *Dorylaimus* spp., *Rotylenchulus* spp. and *Mononchus* spp. (Table 1). However, only the genera *Mesocriconema* spp., *Pratylenchus* spp. and *Meloidogyne* spp. are considered more aggressive in the peach (Walters et al., 2008; Pinochet et al., 1996; Carneiro et al., 1993). Forer et al. (1983) report that *Xiphinema* spp., at high population densities, may contribute to the decline of *Prunus* spp. plants, being associated with the transmission of Tomato Ringspot Virus in peach and cherry trees.

Table 1. Nematodes associated with nine peach rootstocks, expressed for mean nematode abundance in 250 mL of soil (Sa) or 10 g of roots (Ra), relative abundance in the roots and soil (Ra% R and Ra% S) and relative frequency in the roots and soil (Rf% S and Rf% R). Capão do Leão, Rio Grande do Sul, November 2017

Genus	Viamão						Tsukuba						Nemaguard					
	Sa	Ra	Ra%S	Ra%R	Rf%S	Rf%R	Sa	Ra	Ra%S	Ra%R	Rf%S	Rf%R	Sa	Ra	Ra%S	Ra%R	Rf%S	Rf%R
Meloidogyne	407.09	1111.77	100.00	100.00	21.07	57.54	8.50	0.00	60.00	00.00	1.30	0.00	27.45	6.27	36.36	27.27	11.77	2.69
Pratylenchus	6.72	3.27	18.18	18.18	0.35	0.02	29.80	0.30	40.00	10.00	6.06	0.06	9.50	0.09	18.18	9.09	4.09	0.04
Helicotylenchus	303.27	22.09	72.72	72.72	15.69	1.14	369.40	4.40	90.00	20.00	75.18	0.89	128.64	1.63	72.72	9.09	55.19	0.70
Mesocriconema	23.63	30.72	9.09	0.00	1.22	1.59	13.30	3.30	20.00	20.00	2.70	0.67	7.72	1.90	27.27	18.18	3.31	0.82
Trichodorus	5.09	0.00	9.09	0.00	0.26	0.00	12.10	0.30	40.00	10.00	2.46	0.06	8.18	0.54	9.09	9.09	3.51	0.23
Longidorus	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.63	0.00	9.09	0.00	0.70	0.00
Tilenchorchynchus	1.64	0.00	9.09	0.00	0.08	0.00	16.50	1.80	40.00	10.00	3.35	0.36	8.72	0.00	27.27	0.00	3.74	0.00
Mononchus	0.00	0.00	0.00	0.00	0.00	0.00	10.20	0.00	20.00	0.00	2.07	0.00	1.63	0.00	9.09	0.00	0.70	0.00
Hemiciclyophora	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.63	0.00	9.09	0.00	0.70	0.00
Xiphinema	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	5.09	1.64	27.27	9.09	2.18	0.70
Tylenchus	6.09	0.00	27.27	0.00	0.31	0.00	2.10	0.10	10.00	10.00	0.43	0.02	13.09	0.82	27.27	18.18	5.61	0.35
Dorylaimus	5.09	0.00	9.09	0.00	0.26	0.00	10.10	0.12	30.00	10.00	2.05	0.02	3.27	0.12	18.18	9.09	1.40	0.03
Rotylenchulus	2.36	3.27	9.09	9.09	0.12	0.17	7.50	0.00	10.00	0.00	1.53	0.00	3.45	0.00	18.18	0.00	1.48	0.00

Table 1. Continued

Genus	Capdebosq						Umezeiro						Aldrighi					
	Sa	Ra	Ra%S	Ra%R	Rf%S	Rf%R	Sa	Ra	Ra%S	Ra%R	Rf%S	Rf%R	Sa	Ra	Ra%S	Ra%R	Rf%S	Rf%R
Meloidogyne sp.	587.87	1209.92	100.00	100.00	27.83	57.28	35.00	0.00	25.00	0.00	19.90	0.00	74.50	155.33	83.33	83.33	14.06	29.31
Pratylenchus sp.	4.67	0.00	16.66	0.00	0.22	0.00	0.00	0.00	0.00	0.00	0.00	0.00	3.58	0.00	16.66	0.00	0.68	0.00
Helicotylenchus	116.58	22.41	75.00	66.66	5.52	1.06	98.25	0.00	87.50	0.00	55.86	0.00	178.08	48.50	83.33	83.33	33.69	9.15
Mesocriconema	37.33	41.25	58.33	58.33	1.77	1.95	0.00	0.00	0.00	0.00	0.00	0.00	64.08	66.80	16.66	16.66	0.77	1.29
Trichodorus	18.41	10.66	50.00	16.66	0.87	0.50	9.00	0.00	12.50	0.00	5.12	0.00	4.08	0.00	16.66	0.00	0.77	0.00
Longidorus	0.00	0.00	0.00	0.00	0.00	0.00	12.12	0.00	25.00	0.00	6.89	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Tilenchorchynchus	23.16	11.33	58.33	33.33	1.10	0.54	0.00	0.00	0.00	0.00	0.00	0.00	6.50	4.50	16.66	16.66	1.23	0.85
Mononchus	2.33	0.00	8.33	0.00	0.11	0.00	5.75	0.00	25.00	0.00	3.27	0.00	4.50	0.00	8.33	0.00	0.85	0.00
Hemiciclyophora	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Xiphinema	3.75	0.00	16.66	0.00	0.18	0.00	2.37	0.00	12.50	0.00	1.35	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Tylenchus	6.00	1.83	16.66	41.66	0.28	0.08	7.62	0.00	25.00	0.00	4.33	0.00	13.50	7.50	25.00	25.00	2.55	1.41
Dorylaimus	10.41	2.25	41.66	8.33	0.49	0.07	5.75	0.00	12.50	0.00	3.26	0.00	15.83	0.00	33.33	0.00	2.99	0.00
Rotylenchulus	1.75	0.00	8.33	0.00	0.08	0.00	0.00	0.00	0.00	0.00	0.00	0.00	2.58	0.00	8.33	0.00	0.49	0.00

Table 1. Continued

Genus	Okinawa						Flordguard						Industry					
	Sa	Ra	Ra%S	Ra%R	Rf%S	Rf%R	Sa	Ra	Ra%S	Ra%R	Rf%S	Rf%R	Sa	Ra	Ra%S	Ra%R	Rf%S	Rf%R
Meloidogyne	39.83	0.00	50.00	0.00	24.09	0.00	38.84	6.00	76.92	46.15	16.91	2.61	38.22	63.20	64.00	80.00	3.20	3.65
Pratylenchus	2.08	0.00	8.33	0.00	1.26	0.00	0.00	0.00	0.00	0.00	0.00	0.00	3.88	4.80	18.00	18.00	0.32	0.40
Helicotylenchus	99.08	0.00	83.33	0.00	59.92	0.00	112.61	1.38	76.92	7.69	50.04	0.60	161.54	251.52	90.00	90.00	13.52	21.05
Mesocriconema	0.00	0.00	0.00	0.00	0.00	0.00	1.53	0.00	7.69	0.00	0.67	0.00	171.02	365.74	76.00	84.00	25.94	32.24
Trichodorus	2.00	0.00	8.33	0.00	1.20	0.00	11.76	0.00	23.07	0.00	5.12	0.00	17.72	34.80	40.00	40.00	1.48	2.91
Longidorus	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	7.12	0.00	22.00	0.00	0.59	0.00
Tilenchorchynchus	6.00	0.00	16.66	0.00	3.62	0.00	19.23	0.00	46.15	0.00	8.37	0.00	33.20	61.20	64.00	64.00	2.78	5.12
Mononchus	1.75	0.00	8.33	0.00	1.06	0.00	3.46	0.00	15.38	0.00	1.51	0.00	5.06	0.00	14.00	0.00	0.42	0.00
Hemiciclyophora	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Xiphinema	0.00	0.00	0.00	0.00	0.00	0.00	2.92	0.00	15.38	0.00	1.30	0.00	7.54	25.80	22.00	22.00	0.63	2.20
Tylenchus	4.50	0.00	16.66	0.00	2.72	0.00	21.38	2.76	23.07	15.38	9.31	1.21	7.48	15.20	14.00	12.00	0.62	1.27
Dorylaimus	8.00	0.00	16.66	0.00	4.83	0.00	7.69	0.00	23.07	0.00	3.35	0.00	4.56	8.00	12.00	8.00	0.38	0.70
Rotylenchulus	2.08	0.00	8.33	0.00	1.26	0.00	0.00	0.00	0.00	0.00	0.00	0.00	2.00	2.00	6.00	6.00	0.17	0.43

Phytonematodes can reduce the vigour and productivity of a wide range of crops (Anwar et al., 2012; Ngele & Kalu, 2015; Singh & Kumar, 2015). When associated with other factors, they may lead to a decline and early death in stone fruits, especially the peach tree (Forer et al., 1983; Beckman et al., 2008; Walters et al., 2008). Mayer et al. (2015) found that the occurrence of PTSL has been increasing significantly in the peach orchards of Rio Grande do Sul, Brazil in recent decades, attributing part of the effect to the type of rootstock used, soil fertility and the presence of phytonematodes, especially *M. xenoplax*.

Among the nematodes that were identified, the genus *Meloidogyne* spp. was recorded in the roots of six (66.66%) of the nine peach rootstocks under evaluation, whereas in the soil samples, this genus of nematodes was present in the rhizosphere of all nine rootstocks (Table 1). *Meloidogyne* spp. had the highest relative abundance in the soil and roots of the 'Capdeboscq' rootstock, with a mean of 587.87 nem/250 mL soil (SA) and 1209.92 nem/10 g roots (RA), followed by the 'Viamão' rootstock, with a value for SA and RA of 407.09 and 1111.77 respectively (Table 1).

The Aldrighi cultivar had the third largest population abundance for *Meloidogyne* spp., with 74.50 nem/250 mL soil and 155.33 nem/10 g roots (Table 1). The abundance and relative frequency of *Meloidogyne* spp., in the soil and roots followed the same order of magnitude, being greater in 'Capdeboscq', followed by 'Viamão' and 'Aldrighi'.

Comparing the values for mean abundance of *Meloidogyne* spp., in the roots (RA) of the rootstocks, it was found that the Flordaguard and Nemaguard cultivars, considered important sources of resistance to root-knot nematodes (Paula et al., 2011), had a value for RA that was 99% less than that registered in 'Capdeboscq', which is a genotype that is still used as rootstock in some nurseries, but which has been proved to be susceptible to *Meloidogyne* spp. (Fachinello et al., 2000; Paula et al., 2011). Also for *Meloidogyne* spp., it was found that 'Flordaguard' and 'Nemaguard' presented a higher RfS in relation to the RfR, of 84.56% and 77% respectively.

On the other hand, in the susceptible cultivars Capdeboscq, Viamão and Aldrighi, the RfR was higher in relation to the RfS. The presence of this genus of nematode was not recorded in the roots (Ra) of the other rootstocks considered resistant to root-knot nematodes, 'Okinawa', 'Umezeiro' and 'Tsukuba' (Table 1).

Mesocriconema spp., was the second phytoparasite in terms of Sa and Ra. The greatest values for Sa and Ra were recorded in the 'Industry' rootstock (171.01 and 365.75 respectively), followed by the 'Adrighi', 'Capdeboscq' and 'Viamão' rootstocks (Table 1). Although Peach Tree Short Life Syndrome (PTSL) has been attributed to the joint action of biotic, soil and climate factors (Okie et al., 1987), the greater frequency and intensity of damage in the peach has shown a direct relationship to the presence of high populations of the ring nematode (*M. xenoplax*) (Nyczepir et al., 1988). As a consequence, it can be seen that the 'Industry' rootstock, produced from a mixture of the seed of several scions, has greater susceptibility to the ring nematode in relation to the other rootstocks under evaluation.

While *Meloidogyne* spp. displayed greater relative abundance and frequency in the 'Capdeboscq', 'Viamão' and 'Aldrighi' rootstocks in descending order of magnitude, for *Mesocriconema* spp., the greatest abundance and relative frequency were recorded in 'Industry', followed by 'Capdeboscq' and 'Aldrighi'. The 'Viamão' rootstock, although proving to be very susceptible to *Meloidogyne*, showed values for relative abundance and frequency for *Mesocriconema* spp. that were lower than those recorded in the 'Nemaguard' and 'Tsukuba' rootstocks (Table 1).

In the 'Flordaguard', 'Okinawa' and ´Umezeiro' rootstocks, *Mesocriconema* spp. had a value for Sa of 1.53, 0.0 and 0.0 respectively, whereas the Ra was zero in all three rootstocks, suggesting that these cultivars are not preferred hosts (Table 1), compared to the other rootstocks under evaluation.

When comparing the principal phytonematodes that occur associated with the nine *prunus* rootstocks being evaluated, *Pratylenchus* was recorded at a lower frequency and abundance in the soil and roots in relation to *Meloidogyne* and *Mesocriconema*. *Pratylenchus* spp. showed a greater abundance in the soil of the Tsukuba (29.80), Nemaguard (9.50), Viamão (6.72), Capdeboscq (4.87) and Industry cultivars (3.88). On the other hand, the greatest abundance in the roots (RA) was recorded in Industry (4.80) and Viamão (3.27), while in the other genotypes the RA was less than 1.0 or null.

The presence of *Xiphinema* was registered in the rhizosphere of the 'Nemaguard', 'Capdeboscq', 'Umezeiro', 'Flordaguard' and 'Industry' rootstocks, with values for Sa of 1.63, 3.75, 2.37, 2.92 and 7.54 respectively. However, when analysing the roots, the highest value for Ra was recorded in 'Industry' (25.8), followed by 'Nemaguard' (1.64), with *Xiphinema* spp., not being identified in the other rootstocks under evaluation. Specifically in the 'Industry' rootstock, *Xiphinema* had a value for Sa and Ra that were respectively 48.5 and 81.4% higher in relation to *Pratylenchus* spp.

In the rhizosphere and roots of the nine *Prunus* rootstocks under evaluation, most noticeable was the presence of phytoparasitic nematodes of the genera *Meloidogyne* spp. and *Mesocriconema* spp., which are considered the most important in relation to their potential to cause crop damage. Evaluating the incidence of phytonematodes in seven rootstocks in peach orchards in southern Illinois, USA, Walters et al. (2008) reported the occurrence of 11 species belonging to nine genera, with the phytonematodes *Meloidogyne* spp., *Mesocriconema* spp., *Pratylenchus* spp., and *Xiphinema* spp., having the greatest contribution to plant decline and reduced production.

In Rio Grande do Sul, there is still little information on the diversity, and few studies of the population levels of parasitic nematodes in relation to peach rootstock, except for some records of the presence of certain genera, such as *Meloidogyne* spp., and *Mesocriconema* spp. (Carneiro et al., 1993; Rossi, 2002; Gomes et al., 2010; Gomes et al., 2014).

Within the genus *Prunus*, there is great variability for resistance to root-knot nematodes (Pinochet et al., 1996, Esmenjaud et al., 1997; Rossi, 2002; Felipe, 2009; Paula et al., 2011; Souza et al., 2014). Resistance to *Meloidogyne* is relatively easy to transfer by hybridisation and is apparently determined by one or a few dominant genes (Marull et al., 1994), so sources of resistance when available can be used to control the damage caused by this nematode.

Different species of the genus *Meloidogyne* have been considered the most important cause of damage, not only in peach trees (Marull et al., 1994; Pinochet et al., 1996; Gomes et al., 2009; Paula et al., 2011), but also in a wide range of fruit and vegetable species (Anwar et al., 2012; Ngele & Kalu, 2015; Singh & Kumar, 2015). *Meloidogyne* spp. induce the formation of galls in the roots, restricting water and nutrient absorption and plant growth, besides predisposing the plant to attack by other pathogens. In the present study, it was found that

'Capdeboscq', 'Viamão', 'Aldrighi' and 'Industry' are good hosts to this nematode; the use of these rootstocks in areas infested with *Meloidogyne* spp. should therefore be avoided.

Based on the results, it can be seen that the 'Okinawa', 'Tsukuba' and 'Umezeiro' rootstocks displayed low relative abundance and low relative frequency in the soil for *Meloidogyne* spp., while there was no incidence or reproduction of the nematode in the root samples. Although 'Nemaguard' and 'Flordaguard' are sources of resistance to *Meloidogyne*, they have a low Ra compared to the susceptible genotypes. This can be explained by the presence of different alleles that confer variability for resistance on the resistant rootstocks (Marull et al., 1994; Pinochet et al., 1996; Paula et al., 2011), inducing the preferential targeting by parasitic nematodes of the susceptible cultivars ('Capdeboscq', 'Viamão', 'Aldrighi' and 'Industry'), since the plots containing the plants of each rootstock are located side by side, at a spacing of 5.0 × 1.5 m.

Therefore, among the rootstocks under evaluation, the 'Okinawa', 'Tsukuba', 'Nemaguard', 'Flordaguard' and 'Umezeiro' cultivars can be alternatives when planting peach and plum orchards in areas with an incidence of *Meloidogyne* spp. Due to its good adaptation to the climate conditions in Brazil, 'Okinawa' is a good reference source of resistance to *Meloidogyne* spp., which was proven in the present study by the low abundance of this parasite in the soil samples and in the roots. Even so, the 'Okinawa' rootstock is little used in Brazil, but there are high expectations of its use being increased. On the other hand, 'Umezeiro' has also proved to be a good source of resistance to phytopathogenic nematodes, albeit also proving to be graft compatible with only a small number of peach cultivars.

In relation to *M. xenoplax*, studies and sources of resistance are far more scarce compared to those for *Meloidogyne* spp. In the USA, *Mesocriconema* is the main phytonematode associated with PTSL Syndrome. Although PTSL does not have a single cause, an increase in PTSL in the orchards of Rio Grande do Sul, Brazil, and more specifically in the region of Pelotas (the main area of production in the country), is directly related to the type of rootstock being used (Mayer et al., 2006), which is confirmed by the high incidence of phytonematodes in the root system of genotypes that were not selected for use as rootstock, such as the Viamão, Industry, Capdeboscq and Aldrighi cultivars.

Seeds of the Capdeboscq and Aldrighi cultivars were used for a long time in the production of rootstock due to the abundance of stones from the Brazilian canning industry. Capdeboscq seeds are still used in rootstock production because of the high rate of germination, the good graft compatibility of the seedlings with peach and plum cultivars, and the high level of vigour induced in the scions. However, with the development of new scions over the last three decades, waste stones from the peach-canning industry now comprise a mixture of genotypes with high genetic variability and high susceptibility to the different phytonematodes (Bianchi et al., 2014).

The 'Guardian® BY520-9' rootstock has shown good levels of tolerance not only to *Mesocriconema* but also to *Meloidogyne incognita* and *M. javanica* (Okie et al., 1994). However, the search for rootstock resistant to *Mesocriconema*, and with good graft compatibility with *Prunus persica* has not had much success. Studying the relationship between rootstock and PTSL syndrome, Mayer and Ueno (2012) identified a certain level of variability in peach rootstock to PTSL. Currently 'Sharpe®', 'Guardian®' and 'MP-29' are the principal rootstocks with resistance to the ring nematode (*M. xenoplax*) (Okie et al., 1994; Backman et al., 2008; Backman et al., 2012); however, the last two are protected rootstocks and not yet available for use in Brazil. In turn, 'Sharpe®' has shown graft incompatibility and induced poor growth with some Brazilian peach cultivars (unpublished data).

In the present study, although at low population levels, *Xiphinema* spp. and *Pratylenchus* spp. were also identified in the soil and roots of some rootstocks, demonstrating the potential of these phytonematodes to cause economic damage in *Prunus*. *Xiphinema* spp. had a greater value for RA in the 'Industry' rootstock. The incidence of this phytonematode in the peach plants was reported by Walters et al. (2008), and it is among the principal parasitic phytoematodes associated with other fruit trees of the Rosaceae family (Brida et al., 2017; Souza et al., 2017). In turn, *Pratylenchus* spp. had a higher value for RA in the 'Industry' and 'Viamão' rootstocks. Compared to *Meloidogyne* and *Mesocriconema*, the levels of abundance and frequency of *Xiphinema* and *Pratylenchus*, although low, may be an indication that in the near future these nematodes might become another limiting factor associated with the cultivation of stone fruit if measures to contain them, such as the use of resistant rootstocks, are not adopted.

In Europe and North America, the lesion nematodes, *P. penetrans* and *P. vulnus*, are found in high abundance in Rosaceae plantations (Pruyne et al., 1994; Pinochet et al., 1996). In these areas, 'replant problems' have been reported, a disease of complex aetiology that occurs at sites of orchard replanting, involving nematodes, fungi, bacteria and abiotic factors (Dullahide et al., 1994; Walters et al., 2008).

Helicotylenchus spp. was one of the nematode genera with the highest mean value for abundance identified in the soil samples, appearing associated with the root system, especially of the 'Industry' and 'Aldrighi' rootstocks. This nematode is often found in soil samples from several agricultural regions of the world, with a wide geographic distribution, and has been identified in association with several host plants and together with other nematodes, it is also responsible for a decline of the root system (Sharma et al., 1993; Palomares-Rius et al., 2017) there are however, no records of pathogenic importance in the peach tree.

Nematodes of other genera such as *Trichodorus*, *Longidorus Tylenchus*, *Tilenchorchynchus* and *Rotylenchus* have frequently been detected in peach orchards in southern Brazil, but at low population levels. Little is known about their damage to the peach tree, and their pathogenicity to the crop has not yet been confirmed (Gomes & Campos, 2003); in addition, free-living nematodes, such as those of the genus *Mononchus* and *Dorylaimus*, are very common in samples of soil.

Based on the prospecting data, it was evident that in the 'Industry' (a mixture of scions), 'Capdeboscq', 'Aldrighi' and 'Viamão' rootstocks, the incidence of *Mesocriconema* spp. and *Meloidogyne* spp., is high compared to their occurrence recorded in the 'Tsukuba', 'Nemaguard', 'Flordaguard', 'Okinawa' and 'Umezeiro' rootstocks. This data reinforces the need for changes in the production system of *Prunus* rootstocks in Brazil, since according to Mayer et al. (2017), the tradition of using discarded stones from the peach-canning industry has been routine for decades.

The prospecting data and identification of the diversity and population level of phytonematodes in the present study makes an important contribution to guiding the work of genetic improvement of *Prunus* rootstock in Brazil, as well as alerting to the importance of the use of genotypes selected specifically for use as rootstocks. In addition, the data reinforce the need to avoid the use of peach stones discarded by the canning industry in the production of rootstocks.

4. Conclusions

Thirteen genera of nematode were identified associated with the rhizosphere and roots of nine peach rootstocks, including *Meloidogyne* spp., *Mesocriconema* spp., *Pratylenchus* spp., *Xiphinema* spp., *Helicotylenchus* spp., *Trichodorus* spp., *Longidorus* spp., *Tylenchus* spp., *Tilenchorchynchus* spp., *Rotylenchus* spp., *Mononchus* spp., *Dorylaimus* spp. and *Hemiciclyophora* spp.

The genera *Meloidogyne* spp. and *Mesocriconema* spp. were found at a higher population level in all the rootstocks under evaluation.

The 'Okinawa' and 'Umezeiro' rootstocks did not present any population levels of *Meloidogyne* spp. or *Mesocriconema* spp.

The soil and root samples of the 'Industry' rootstock displayed a high abundance and frequency of *Mesocriconema* spp., and therefore should not be recommended for use in rootstock production.

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