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ORIGINAL ARTICLE

Plant parasitic nematode communities associated to apple orchards in the Southern Brazil

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Abstract

In recent years, apple (*Malus domestica* Borkh) has been one the most important fruit crops in the southern part of Brazil. Despite this, research about the attack by plant-parasitic nematodes (PPNs) and/or its involvement with apple replant disease (ARD) is poorly known up to now. Our study aimed to (*i*) identify and quantify the major PPNs (*morphological groups*) infesting apple growing areas in southern of Brazil; (*ii*) to evaluate the relationship between altitude, bioclimatic variables, types of soil and the occurrence and abundance of PPNs; and (*iii*) to characterize (morphologically and morphometrically) the *Pratylenchus* species obtained from the apple orchards. During 2020/2021 crop season, we identified five genera of PPNs with variable abundance *Pratylenchus* (95%; 50 to 425 specimens/250 cm^3 soil), *Helicotylenchus* (95%; 25 to 875 specimens/250 cm^3 soil), *Tylenchus* (90%; 25 to 325 specimens/250 cm^3 soil), *Xiphinema* (90%; 25 to 550 specimens/250 cm^3 soil) and *Mesocriconema* (21%; 25 to 250 specimens/250 cm^3 soil). The ecological indices were reasonably high, with values varying from 0.78 to 1.35 for *H′* and 0.63 to 0.98 for *J*. The annual mean temperature (BIO1) and annual precipitation (BIO12) strongly influenced the abundance values, albeit in different ways ($p < 0.01$). Nevertheless, there was no influence of bioclimatic variables in the distribution of PPNs. *Pratylenchus zeae* and *P. penetrans*, which had not been reported in the apple plants in Brazil, were identified associated with the crop. Our findings open new perspectives about the research towards management measures of PPNs in infested apple orchards (nematicide development and selection of resistant rootstocks), especially where the ARD is already present. Naturally, epidemiological issues, such as delimitation of risk areas, should be taken into account as well.

Keywords *Pratylenchus* · Epidemiology · *Malus domestica* · Apple replant disease · Soil sickness

Introduction

Apple (*Malus domestica* Borkh) is a fruit crop with a high economic importance for the southern of Brazil (Fioravanço and Santos 2013). Like other fruits pome trees, damage and losses tend to occur over time, which may be related to the increase in the soil pathogens populations (Lü and Wu 2018; Yim et al. 2013). Under this aspect, the known "apple replant disease" (ARD) occurs worldwide in areas intended for replanting apple trees and/or closely related fruit pome species (Winkelmann et al. 2019), and the symptoms include underdevelopment, uneven growth, discoloured roots and often losses (Mahnkopp et al. 2018). The characterization of ARD is usually a plenty complex labour, requiring the adoption of new approaches to solve the problem (Mazzola and Manici 2012; Winkelmann et al. 2019).

Historically, the ARD has been attributed to abiotic and biotic components, such as oomycetes, fungi and several plant parasitic nematodes (PPNs) (Van Schoor et al. 2009). For instance, Wallace and MacDonald (1979) state that several PPNs can cause damages directly to apple trees, such as *Xiphinema americanum* Cobb, 1913; *Hoplolaimus galeatus*

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(Cobb, 1913) Thorne, 1935; *Pratylenchus penetrans* Cobb, 1917; and *Helicotylenchus* Steiner, 1945. Root lesion nematodes, *Pratylenchus* species, has received more attention as a putative pathogenic agent, mostly *P. penetrans*, including for its involvement with the establishment of ARD (Tewoldemedhin et al. 2011). In Brazilians apple orchards, despite the occurrence of ARD (Boneti et al. 1999) and the predominance of susceptible rootstocks in traditional growing regions (Rufato et al. 2021), surveys have not been carried out recently, resulting in only some records (Dias-Arieira et al. 2010). The lack of the knowledge about the nature this soilborne syndrome not only makes the management a fruitless task (Deakin et al. 2019), but also can lead to the replacement of numerous areas of cultivation. Therefore, knowledge about the nature of the ARD and PPNs occurring in Brazilian orchards becomes a crucial issue.

In view of the above, we aimed in this work (*i*) to identify and quantify the major PPNs associated with apple plants in growing areas in the states of Paraná and Rio Grande do Sul; (*ii*) to investigate the relationships between altitude, bioclimatic variables and soil types and PPNs (incidence and abundance); and (*iii*) to characterize (morphologically and morphometrically) the populations of *Pratylenchus*. This is the first study on the occurrence of PPNs associated with apple orchards in the state of Paraná and the relationships between PPNs with soil type, altitude and bioclimatic variables.

Material and methods

Samples collection, specimen extraction, diversity indices and concepts used

During the 2020/2021 season, sampling (soil and roots) was undertaken across 19 growing apple areas located in Paraná and Rio Grande do Sul states, Brazil (Table 1). Each sample was composed of 10 subsamples, collected arbitrarily (*zig zag*), at a depth of 0–25 cm (rhizosphere) from apple trees throughout the orchard. Exceptionally, the root samples were not collected in Palmas– PR (Labnema 40), because the seedlings were very young. The samples were subsequently transported to the Nematology Laboratory of the Federal University of Pelotas (Rio Grande do Sul, Brazil) for nematode extraction and identification.

The extraction of nematodes from the soil was carried out by the flotation-centrifugation method proposed by Jenkins (1964). For this, 250 cm^3 aliquots were homogenized and

Table 1 Municipality, geographical coordinates, altitude, *Pratylenchus* species, rootstocks and soil types of the samples collected in the states of Paraná and Rio Grande do Sul

Municipality (States)	Codes	Geographical Coordinates		Altitude (m)	*Pratylenchus* species	Rootstocks[a]	Soil Types[b]
		Latitude	**Longitude**				
Palmas (PR)	Labnema38	−26.47102	−51.98014	1105	*P. penetrans*	M7	Cambisol
Palmas (PR)	Labnema39	−26.47102	−51.98014	1105	*P. zeae*	M9	Cambisol
Palmas (PR)	Labnema40	−26.52640	−52.03179	1084	-	M9	Cambisol
Palmas (PR)	Labnema41	−26.52640	−52.03179	1084	*P. zeae*	M9	Cambisol
Palmas (PR)	Labnema42	−26.52352	−51.99708	1123	*P. penetrans*	M9	Cambisol
Palmas (PR)	Labnema43	−26.52352	−51.99708	1123	*P. zeae*	Marubakaido	Cambisol
Palmas (PR)	Labnema44	−26.52352	−51.99708	1123	*P. zeae*	M9	Cambisol
Palmas (PR)	Labnema45	−26.51364	−51.93928	1084	*P. penetrans*	M9	Cambisol
Palmas (PR)	Labnema46	−26.51295	−51.94582	1084	*P. zeae*	M9	Cambisol
Palmas (PR)	Labnema47	−26.51492	−52.01866	1083	*P. zeae*	M9	Cambisol
Palmas (PR)	Labnema48	−26.57793	−51.92203	1077	*P. penetrans*	Marubakaido	Cambisol
Palmas (PR)	Labnema49	−26.57793	−51.92203	1077	*P. zeae*	M9	Cambisol
Palmas (PR)	Labnema50	−26.56091	−51.61235	1308	*Pratylenchus*	Marubakaido	Cambisol
Palmas (PR)	Labnema51	−26.56031	−51.61525	1299	*P. penetrans*	Marubakaido	Cambisol
Palmas (PR)	Labnema52	−26.56138	−51.60808	1297	*P. zeae*	Marubakaido	Cambisol
Guarapuava (PR)	Labnema53	−25.56557	−51.56392	1068	*P. zeae*	M9	Cambisol
Guarapuava (PR)	Labnema54	−25.56557	−51.56392	1068	*P. zeae*	M9	Cambisol
Arroio do Padre (RS)	Labnema55	−31.41073	−52.44334	187	*P. zeae*	M9	Alisol
Pelotas (RS)	Labnema56	−31.41555	−52.46609	286	*P. zeae*	M7	Alisol

[a]Rootstocks: M7 (*M. pumila* Miller), M9 (*M. pumila*) and Marubakaido (*M. prunifolia*)

[b]Classification of types of soil obtained from *World Reference Base for Soil Resources* (WRB), universal system recognized by *International Union of Soil Science* (IUSS) and FAO

the resulting suspension was subjected to sieving (20 and 400 mesh) and centrifugation (1800 rpm) in sucrose solution. For roots, the methodology adopted was crushing followed by flotation-centrifugation described by Coolen and D'Herde (1972). Thus, 10 g of roots, previously washed and crushed, was subjected to sieving (20 and 500 mesh) and centrifugation (1800 rpm). Nematodes were also extracted with sucrose solution (Machado et al. 2019).

Estimates of population densities were obtained from counts performed under an optical microscope (40× and 100×) using Peters' slides, and the identification of PPNs (*morphological groups*) was carried out through the observation of morphological characters (Mai and Mullin 1996). In our study, incidence (%) indicates the percentage of the number of samples with a given taxon (Genus) over the total number of samples (soil and/or roots) (Simon et al. 2018). Estimates of population densities — either in soil (specimens per 250 cm^3) and/or roots (specimens per 10 g) — will be referred as abundance (Márquez et al. 2021). In addition, we obtained for each orchard, the Shannon–Weaver diversity index ($H' = -\Sigma p_{i=1} \log_b p_i$, where p_i is the proportion of the genera i) and Pielou's evenness ($J = H'/\log S$; J varying between 0 and 1), using the Vegan 2.5–2 package (Oksanen 2018) in the R version 4.1.1 (R Development Core Team 2021).

Relationships between plant parasitic nematodes and altitude, bioclimatic variables and types of soil

All statistical analyses were performed using software R4.1.1 (R Development Core Team 2021) and various packages, such as sdm (Naimi and Araújo 2016), raster (Hijmans et al. 2020) and dismo (Hijmans et al. 2017). From the geographic coordinates (longitude and latitude) obtained for each sampled area, the soil type using the HWSD — Viewer software (FAO 2022) and 19 climatic variables (Table 2) included in the WordClim database (https://www.worldclim.org/) (Fick and Hijmans 2017) was determined. Due to its biological significance for PPNs, the predictor variables BIO1 and BIO12 were included a priori (Márquez et al. 2021) in the full model while the other variables were selected, avoiding collinearity, based on the respective variance inflation factor values (variance inflation factor — VIF < 10.0) (Dormann et al. 2013). Then, generalized linear models (GLMs), involving soil types, altitude and climate variables and PPNs, were selected according to the Akaike information criterion (AIC) and VIFs. The selected GLMs for each variable (PPNs, incidence and abundance) were then subjected to analysis of variance (ANOVA).

Table 2 Bioclimatic variables used in Plant Parasitic Nematodes Distribution Models extracted from the WordClim dataset (www.worldclim.org/bioclim)

Bioclimatic variables	Descriptions	Unit
BIO1	Annual mean temperature	mm
BIO2	Mean diurnal range (mean of monthly (maximum temperature − minimum temperature))	° C
BIO3	Isothermality (BIO2/BIO7) (× 100)	° C
BIO4	Temperature seasonality (standard deviation of temperatures × 100)	° C
BIO5	Maximum temperature of warmest month	° C
BIO6	Minimum temperature of coldest month	° C
BIO7	Temperature annual range (BIO5-BIO6)	° C
BIO8	Mean temperature of wettest quarter	° C
BIO9	Mean temperature of driest quarter	° C
BIO10	Mean temperature of warmest quarter	° C
BIO11	Mean temperature of coldest quarter	° C
BIO12	Annual precipitation	mm
BIO13	Precipitation of wettest month	mm
BIO14	Precipitation of driest month	mm
BIO15	Precipitation seasonality (coefficient of variation)	mm
BIO16	Precipitation of wettest quarter	mm
BIO17	Precipitation of driest quarter	mm
BIO18	Precipitation of warmest quarter	mm
BIO19	Precipitation of coldest quarter	mm

Morphological and morphometric characterization of *Pratylenchus* populations infesting apple orchards in Paraná and Rio Grande do Sul states

Due to be the most important genus for the apple crop (Mazzola and Mullinix 2005; Tewoldemedhin et al. 2011), we focus in the characterization of *Pratylenchus*. For these purposes, PPN populations obtained from apple orchards were maintained in sorghum (*Sorghum bicolor* (L.) Moench) 'BRS 506' and tomato (*Lycopersicon esculentum* Mill.) 'Santa Clara' and 'Santa Cruz' plants under greenhouse conditions.

The specimens were extracted using the method of Coolen and D'Herde (1972) and by Jenkins (1964) for roots and soil, respectively. Temporary slides (fixed on formalin) were prepared, and corresponding photomicrographs were obtained using the Eclipse E200 vertical microscope and, finally, measurements were performed using the BEL VIEW 7.1 and Digimizer version 5.7.2 (Barsi 2021). The following characters were measured for females and, when applicable, males: body length (*L*), greater body width (*W*), distance from the opening of the dorsal oesophageal gland to the style basal nodes (*DEGO*), stylet length (*St*), distance from the labial region to the vulva (*DLV*), distance between the vulva and anus (*DVA*), spicule length (*Sl*) (males), tail length (*Tl*) and body width in the anal region (*Aw*). In addition, the indices of Man *a* (*L*/*W*), *c* (*L*/*Tl*), *c'* (*Tl*/*Aw*) and *V*% [(*DLV*/*L*) × 100] were calculated. In addition, the number of labial rings, the shape of the stylet bulb and the morphology of the tail were observed. The characterization of the species was based mainly on Castillo and Vovlas (2007) and Gonzaga (2006).

We carried out the hierarchical clustering on principal component analysis (HCPCAs) using FactorMineR package (Husson et al. 2020). Our measurement averages (*L*, *St*, *V* and *Tl*) and other observations (presence (1) or absence (0) of males) were compared with data of *Pratylenchus* species published on previous works (Román and Hirschmann 1969, Café-Filho and Huang 1988, Torres et al. 2004, Torres et al. 2015, Gonzaga 2006, Siqueira 2007, Kumari 2012, Janssen et al. 2017, Mokrini et al. 2016, Flis et al. 2018, Li et al. 2019). This analysis was performed using R version 4.1.1 (R Development Core Team 2021).

Results

Survey of plant parasitic nematodes in apple orchards in the states of Paraná and Rio Grande do Sul

The ecological indices were reasonably high, with values varying of 0.78 to 1.35 for *H'* and 0.63 to 0.98 for *J*. The abundance values for PPNs were quite variable (150–1325 individuals/250 cm^3 of soil). Five genera of PPNs belonging to five families (Hoplolaimidae, Pratylenchidae, Criconematidae, Tylenchidae and Longidoridae) were identified, namely *Helicotylenchus*, *Pratylenchus*, *Mesocriconema*, *Tylenchus* and *Xiphinema*. The genus *Pratylenchus* showed wide distribution, with incidence of 95% and abundance ranging from 50 to 425 individuals/250 cm^3 (soil) and 150 to 8525 individuals/10 g (roots). The incidence of *Helicotylenchus* was 95% of the soil (25 to 875 individuals/250 cm^3) followed by *Xiphinema*, almost 90% of the areas (25 to 550 individuals/250 cm^3 soil) and *Tylenchus* found in 90% of areas (25 to 325 individuals/250 cm^3 soil; 25 to 100 individuals/10 g roots). The incidence of *Mesocriconema* was the lowest (21%), ranging between 25 and 250 individuals/250 cm^3 (soil) and 25 individuals/10 g (roots).

Relationships between plant parasitic nematodes and altitude, bioclimatic variables and types of soil

For each response variable, the selected GLMs and VIFs values are shown in the Table 3. The total number of PPNs was influenced by the variables BIO12 (negative), BIO8 (negative) and BIO7 (positive) ($p < 0.01$). For each genus, BIO1 negatively influenced the abundance of *Helicotylenchus*, *Xiphinema* and *Tylenchus*, but positively for *Mesocriconema* ($p < 0.01$). The variable BIO12 negatively affected the abundance values for *Helicotylenchus*, *Pratylenchus* and *Tylenchus* and positively for *Xiphinema* ($p < 0.01$). In relation to BIO8, there was a negative influence for *Pratylenchus* and *Mesocriconema* and a positive influence for *Helicotylenchus*. The variables BIO7 and BIO4 only influenced *Helicotylenchus* (positive relationship) and *Tylenchus* (negative relationship) ($p < 0.01$). Conversely, there was not significant interaction for incidence of no genus of PPNs. Furthermore, the altitude and types of soil did not influence either studied genus of PPNs.

Morphological and morphometric characterization of *Pratylenchus* populations infesting apple orchards in Paraná and Rio Grande do Sul states

Pratylenchus zeae (67%) and *P. penetrans* (28%) were identified in the sampled orchards (Table 4). Unfortunately, one population of *Pratylenchus* (5%; Labnema 40) had insufficient information (few specimens) for secure identification. The identification of *P. zeae* was initially based on the morphology and absence of males. The specimens showed three labial rings, and the stylet bulbs showed a broad and flat base, as described before (Castillo and Vovlas 2007; Roman and Hirschmann 1969; Kolombia et al. 2020). The most specimens showed pointed tail while few individuals showed a subacute tail with smooth terminal, as stated by

Table 3 Generalized linear models (GLMs) used to examine the relationships between environmental variables, abundance and incidence of plant parasitic nematodes (PPNs) for each genus (*Pratylenchus* (*Praty*), *Helicotylenchus* (*Helico*), *Tylenchus* (*Tylen*), *Xiphinema* (*Xiphi*), *Mesocriconema* (*Meso*))

Models	Global model	Chosen model	Link function	VIF
1	PPNs ~ 1 + BIO1 + BIO12 + BIO7 + BIO8	PPNs ~ 1 − BIO12*** + BIO7*** − BIO8***	Poisson (log)	2.42; 2.46; 1.45
2	*Helico* ~ 1 + BIO1 + BIO12 + BIO7 + BIO8	*Helico* ~ 1 − BIO1*** − BIO12*** + BIO7*** + BIO8***	Poisson (log)	26.63; 69.93; 20.07; 14.43
3	*Praty* ~ 1 + BIO1 + BIO12 + BIO8	*Praty* ~ 1 − BIO12*** − BIO8***	Poisson (log)	1.06; 1.06
4	*Xiph* ~ 1 + BIO1 + BIO12	*Xiph* ~ 1 − BIO1*** + BIO12***	Poisson (log)	1.09; 1.09
5	*Tylen* ~ 1 + BIO1 + BIO12 + BIO3 + BIO4	*Tylen* ~ 1 − BIO1*** − BIO12*** − BIO4***	Poisson (log)	1.23; 1.20; 1.07
6	*Meso* ~ 1 + BIO1 + BIO12 + BIO8	*Meso* ~ 1 + BIO1*** − BIO8***	Poisson (log)	1.26; 1.26
7	*IncidPraty* ~ 1 + BIO1 + BIO12 + BIO4 + BIO8	*IncidPraty* ~ 1 − BIO12^{ns}	Binomial (logit)	-
8	*IncidHelico* ~ 1 + BIO1 + BIO12 + Solo + BIO3 + BIO4	*IncidHelico* ~ 1	Binomial (logit)	-
9	*IncidTylen* ~ 1 + BIO1 + BIO12 + altitude	*IncidTytlen* ~ 1 + BIO1^{ns} − BIO12^{ns} + altitude^{ns}	Binomial (logit)	3.50; 1.65; 3.93
10	*IncidXiph* ~ 1 + BIO1 + BIO12 + BIO3 + BIO4 + BIO7 + BIO8	*IncidXiph* ~ 1 + BIO3 *IncidXiph* ~ 1 + BIO4 *IncidXiph* ~ 1 + BIO7 *IncidXiph* ~ 1 + BIO8	Binomial (logit)	-
11	*IncidMeso* ~ 1 + BIO1 + BIO12	*IncidMeso* ~ 1	Binomial (logit)	-

BIO1 (annual mean temperature); BIO2 (mean diurnal range (mean of monthly [maximum temperature − minimum temperature])); BIO5 (maximum temperature of warmest month); BIO6 (minimum temperature of coldest month); BIO7 (temperature annual range (BIO5–BIO6)); BIO8 (mean temperature of wettest quarter); BIO9 (mean temperature of driest quarter); BIO12 (annual precipitation); BIO15 (precipitation seasonality (coefficient of variation)); BIO19 (precipitation of coldest quarter)

Incid incidence, *VIF* variance inflation factors

(−): absent; (+) positive effect; (−) negative effect

$^{***}p < 0.01$; $^{ns}p > 0.01$

Castillo and Vovlas (2007) and Gonzaga (2006). Our measurements (*L*, *St*, *Tl*, *a*, *c* and *V*%) were very close to the results previously described (Doucet and Cagnolo 1998; Roman and Hirschmann 1969) being the *V*% and *St* values ranging between 75 and 77% and 14.5 and 15.3 µm, respectively. The *DGO* values were consistent with those observed by Roman and Hirschmann (1969), ranging between 1.8 and 3.0 µm.

In the populations of *P. penetrans* were initially observed the presence of males (1–3). For females, we observed the labial region slightly detached (*off set*) from the body and three labial rings (Roman and Hirschmann 1969; Castillo and Vovlas 2007). The stylet bulb shape was quite rounded and/or well separated and concave, and, less frequently, the bulbs were directed laterally. Similarly, Castillo and Vovlas (2007) and Tarte and Mai (1976) stated that *P. penetrans* females may have very rounded or shell-shaped bulbs in the anterior region. High variability was observed for morphometric data, as described by Janssen et al. (2017) to *L*, *St*, *DLV*, *W*, *Aw*, *Tl*, *DVA*, and the Man indices: *a*, *c*, *c′* and *V*%. The average *L* (454.0–516.3 µm) was similar to several descriptions in the literature (Loof 1960; Ryss 1988; Sher and Allen 1953; Taylor and Jenkins 1957) as well as *DGO* (2.1–2.5 µm) (Roman and Hirschmann 1969). Values obtained for *V*% (76–82%) were congruent with the information collected by Gonzaga (2006). The Man indices mostly resembled the information obtained by Loof (1960) and Ryss (1988), and sometimes, the *St* (13.9–16.1 µm) values as well. To males, our major measurements were *L* (382.48–539.99 µm), *W* (18.21–26.49 µm), *St* (13.04–15.28 µm), *DEGO* (1.86–2.85 µm), *a* (18.26–23.45), *c* (18.97–33.95), *Tl* (14.40–27.91 µm) and *Sl* (9.61–13.96 µm).

The first two components accounted for about 72% of the variance and from HCPCAs (Fig. 1A), and the *Pratylenchus* populations (operational taxonomic units — OTUs) were grouped into three conspicuous clusters, as follows: (*i*) The first cluster was composed by *P. neglectus* and all *P. brachyurus* populations (100% of correspondence); (*ii*) the second cluster with *P. scribneri*, and all *P. zeae* populations (100% of correspondence); and (*iii*) the last cluster formed by *P. vulnus*, *P. coffeae*, *P. fallax*, *P. pseudofalax*, Labnema 50 and all *P. penetrans* populations (100% of correspondence) (Fig. 1B).

Discussion

Notwithstanding the records of the association of several PPNs with apple orchards worldwide, potential pathogenic is often attributed to *Pratylenchus* species (Castillo and Vovlas 2007). For instance, Seinhorst (1998) estimated that the threshold of tolerance to *P. penetrans-Malus* interaction is just of 1.5 specimens/g of soil, including values close or lower those recorded here. Overall, our findings are mostly aligned with those described previously,

Table 4 Morphometry of *Pratylenchus* species (females) identified in samples from apple orchards in the states of Paraná and Rio Grande do Sul

Species	Codes	*n*	*L* (µm)	*DEGO* (µm)	*St* (µm)	*a*	*c*	*c′*	*V* (%)
P. penetrans	Labnema38	10	516.3[a] ± 59.83[b] (394.8–628.7)[c]	2.4 ± 0.40 (2.1–2.8)	14.9 ± 0.94 (13.3–16.0)	20.6 ± 1.47 (18.2–22.8)	19.3 ± 2.87 (13.6–23.0)	1.8 ± 0.28 (1.2–2.0)	81 ± 0.02 (77–83)
P. penetrans	Labnema42	9	454.0 ± 80.64 (363.0–66.8)	2.50 ± 0.29 (2.0–3.1)	14.90 ± 0.60 (14.0–15.9)	21.30 ± 2.28 (17.1–25.2)	15.50 ± 2.77 (11.5–21.0)	2.20 ± 0.42 (1.7–2.6)	76 ± 0.03 (75–81)
P. penetrans	Labnema45	10	506.5 ± 23.70 (470.7–527.9)	2.4 ± 0.28 (2.1 – 2.9)	15.2 ± 0.73 (14.3 – 16.1)	20.5 ± 1.50 (19.1– 22.1)	15.5 ± 1.36 (13.6 – 17.1)	2.3 ± 0.30 (1.9 – 2.9)	77 ± 0.01 (76 – 79)
P. penetrans	Labnema48	10	497.5 ± 36.53 (426.0–527.8)	2.5 ± 0.26 (2.1–2.10)	14.6 ± 0.60 (13.9–17)	20.6 ± 1.16 (18.9–21.10)	17.0 ± 2.32 (14–19.7)	2.1 ± 0.41 (1.5–2.8)	76 ± 0.06 (63–84)
P. sp	Labnema50	10	476.3 ± 32.01 (431.8–530.9)	2.1 ± 0.49 (1.4–2.8)	14.4 ± 0.90 (12.5–15.7)	20.6 ± 2.10 (16.7–22.1)	17.8 ± 2.58 (15.2–18.5)	1.9 ± 0.38 (1.2–2.4)	77 ± 0.03 (73–82)
P. penetrans	Labnema51	10	512.0 ± 28.67 (481.1–557.5)	2.2 ± 0.44 (1.7–2.7)	14.7 ± 0.68 (13.9–15.6)	19.2 ± 1.80 (17.3–22.0)	17.6 ± 3.02 (12.6–22.9)	2.0 ± 0.38 (1.7–2.7)	80 ± 0.02 (76–82)
P. zeae	Labnema39	10	509.6 ± 21.75 (474.5–531.2)	2.2 ± 0.29 (1.7–2.8)	14.7 ± 0.70 (14.0–15.9)	20.6 ± 1.22 (19.0–23.2)	17.2 ± 2.70 (14.6–23.4)	2.1 ± 0.33 (1.6–2.7)	77 ± 0.02 (74–80)
P. zeae	Labnema41	5	494.6 ± 68.30 (453.2–614.2)	2.5 ± 0.22 (2.3–2.8)	14.8 ± 0.49 (14.1–15.5)	20.7 ± 1.73 (18.6–23.4)	16.9 ± 0.64 (16.3–17.8)	1.8 ± 0.10 (1.7–2.0)	79 ± 0.05 (78–86)
P. zeae	Labnema43	10	490.6 ± 47.93 (417.4–570.8)	2.8 ± 0.40 (2.4–3.0)	14.6 ± 0.80 (13.3–15.6)	21.9 ± 1.85 (19.2–26.1)	15.9 ± 2.29 (14.0–21.0)	2.2 ± 0.31 (1.6–2.5)	76 ± 0.03 (70–78)
P. zeae	Labnema44	10	534.6 ± 68.42 (399.0–596.9)	2.5 ± 0.40 (1.8–3.1)	15.1 ± 0.73 (13.5–15.5)	21.6 ± 1.80 (20.0–23.2)	16.9 ± 1.12 (15.9–18.7)	1.9 ± 0.23 (1.4–2.2)	77 ± 0.01 (75–79)
P. zeae	Labnema46	10	482.0 ± 50.65 (433.3–582.4)	2.3 ± 0.38 (1.8–2.8)	15.0 ± 0.77 (13.3–15.8)	20.5 ± 0.99 (18.8–22.2)	16.4 ± 2.33 (12.9–20.3)	2.1 ± 0.31 (1.8–2.8)	76 ± 0.02 (71–78)
P. zeae	Labnema47	10	486.1 ± 50.26 (397.7–595.3)	2.5 ± 0.44 (1.8–2.9)	14.6 ± 0.54 (13.8–15.4)	20.5 ± 1.31 (18.2–22.8)	15.4 ± 2.08 (13.9–17.7)	2.2 ± 0.27 (1.8–2.5)	75 ± 0.03 (71–79)
P. zeae	Labnema49	10	504.1 ± 41.94 (435.4–555.6)	2.6 ± 0.27 (2.2–3.0)	14.8 ± 0.70 (13.8–16.2)	19.7 ± 1.65 (17.1–22.5)	16.9 ± 2.04 (14.2–20.1)	2.0 ± 0.21 (1.8– 2.4)	77 ± 0.01 (75–78)
P. zeae	Labnema52	10	525.7 ± 25.62 (483.0–568.5)	2.2 ± 0.31 (1.7–2.6)	14.5 ± 1.09 (12.2–16.2)	21.8 ± 1.70 (19.2–24.3)	18.1 ± 2.39 (15.1–22.7)	2.2 ± 0.30 (1.7–2.6)	76 ± 0.02 (73–79)
P. zeae	Labnema53	10	517.7 ± 58.35 (454.8–609.5)	2.2 ± 0.38 (1.7–2.8)	15.3 ± 0.46 (14.3–15.9)	21.9 ± 1.43 (19.5–24.8)	17.8 ± 3.28 (14.7–18.6)	2.2 ± 0.27 (1.7–2.5)	76 ± 0.01 (74–78)
P. zeae	Labnema54	10	528.0 ± 88.47 (380.0–661.0)	2.0 ± 0.40 (1.3–2.8)	14.6 ± 0.98 (12.8–15.6)	20.3 ± 2.06 (17.8–23.7)	18.0 ± 3.36 (13.4–26.1)	1.9 ± 0.35 (1.4–2.5)	76 ± 0.02 (71–79)
P. zeae	Labnema55	10	478.2 ± 17.60 (460.3–517.7)	2.1 ± 0.15 (1.8–2.3)	14.5 ± 0.60 (13.8–15.4)	20.4 ± 1.33 (17.6–22.0)	16.3 ± 0.84 (14.9–17.7)	1.9 ± 0.14 (1.7–2.2)	76 ± 0.02 (73–78)
P. zeae	Labnema56	10	469.5 ± 35.64 (410.9–525.0)	2.1 ± 0.30 (1.8–2.6)	14.7 ± 0.47 (13.7–15.5)	23.7 ± 2.36 (19.8–27.7)	16.6 ± 1.42 (13.2–18.4)	2.4 ± 0.29 (2.0–2.8)	77 ± 0.01 (76–79)

n number of specimens (females), *L* body length, *DEGO* distance from the opening of the dorsal oesophageal gland to the style basal nodes, *St* stylet length, *V* (%) distance of the anterior region to vulva, *a L/W*, *c L/Tl*, *c′ L/Aw*

[a]Mean

[b]Standard deviation

[c](Min–max)

including the predominance of *Pratylenchus* species in apple orchards. In the past, *P. scribneri* was detected parasitizing young apple plants collected in Vacaria (RS) (Monteiro et al. 1987), whereas *P. zeae* Graham, 1951, and *P. pseudofallax* were found associated with *M. silvestris* in Veranópolis and Pelotas, RS (Café-Filho and Huang 1988; Café-Filho and Huang 1989). More recently, Dias-Arieira et al. (2010) found *P. brachyurus* associated to apple orchards in the state of Paraná in Alto Piquiri, PR. To our knowledge, there is no former reports of *P. penetrans* associated to apple trees in Brazil.

Although none of the sampled areas has been previously sampled to determine the PPNs infestations, it is possible that ARD is already present in some sites, since that in almost all orchards had plants with different ages, indicating that the replacement was taken on over the time. Unfortunately, little attention has been driven to PPNs by technicians involved with apple's growers in Brazil instead of what happens in other countries of the world. Also, the type of rootstock does not seem to have restricted *P. penetrans* infection, occurring on M7 (*M. pumila* Miller), M9 (*M. pumila*) and Marubakaido (*M. prunifolia*). Indeed,

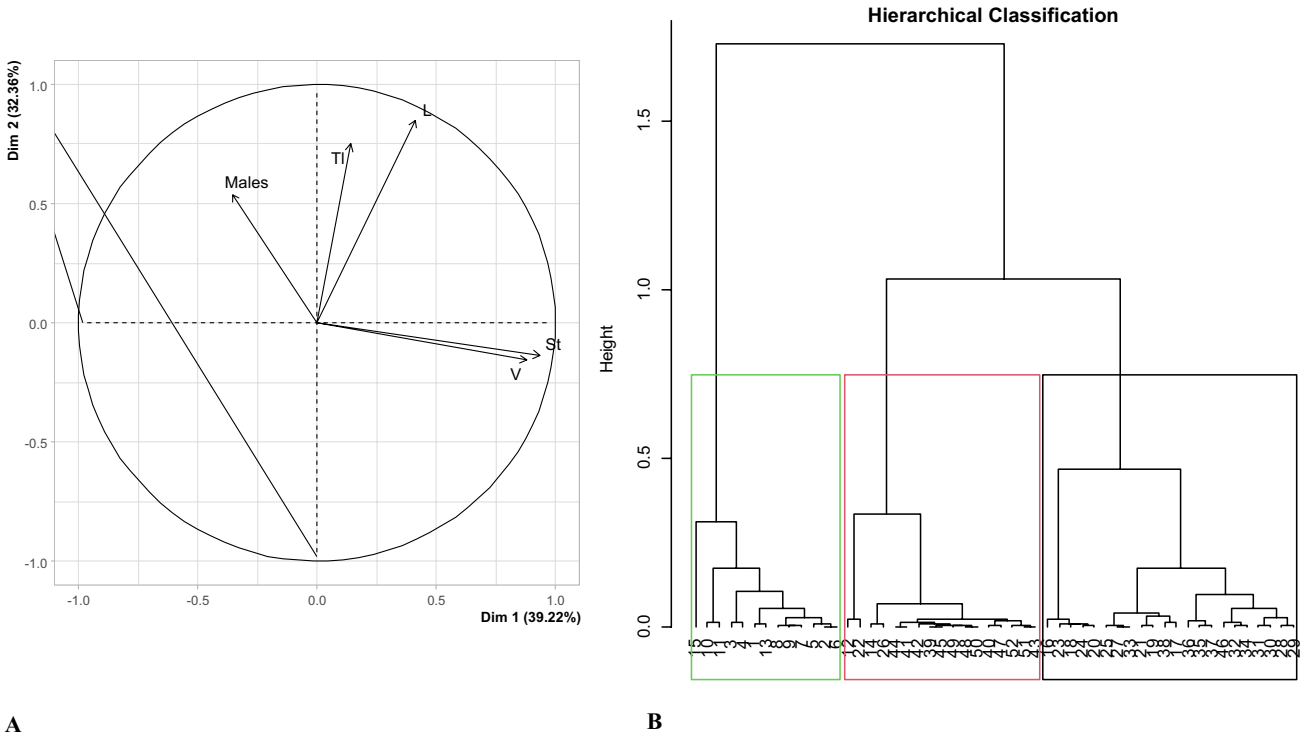

Fig. 1 Variable graphics (**A**) and dendrograms (**B**) obtained from morphometric data of *Pratylenchus* populations subjected to hierarchical clustering based on principal component analysis (PCA). **A** *L* (body length), *Tl* (tail length), *St* (stylet length) and *V*% were used for PCA. **B** Individuals of *P. zeae* (Pz; 12, 22, 39–52), *P. brachyurus* (1–11, 13), *P. coffeae* (20, 21), *P. neglectus* (15), *P. vulnus* (18, 19), *P. scribneri* (14, 26), *P. fallax* (31), *P. pseudofalax* (32), *P. penetrans* (*Pp*; 16,17, 23–25, 27–30, 33–38) and Labnema 50 (46). Our data: *Pp* (33–38), *Pz* (39–52) and Labnema 50. Other data were obtained from Román and Hirschmann (1969), Café-Filho and Huang (1988), Torres et al. (2004), Torres et al. (2015), Gonzaga (2006), Siqueira (2007), Kumari (2012); Janssen et al. (2017), Mokrini et al. (2016), Flis et al. (2018) and Li et al. (2019)

little attention has been given to the use of resistant or tolerant rootstocks, such as the CG (Cornell-Geneva) series (Isutsa and Merwin 2000), which still are not widespread in Brazil. Furthermore, more awareness should also be directed towards cultural tools, such as the use of pre-planting non-hosts in established orchards and/or nurseries (Kanfra et al. 2021).

In relation to the factors driving PPNs structure, our results are, at least partially, in according to with those described previously, in which annual precipitation (BIO12) negatively influenced PPNs populations (Hamza et al. 2018), while increasing of the annual mean temperature (BIO1) did not result in significant interference. The negative relationship of PPNs genera (*Helicotylenchus*, *Xiphinema* and *Tylenchus*) with BIO1-confirmed studies performed in other crops (Marquez et al. 2021). Collectively, these results allow us to theorize about current risk areas (like regions with less rainfall) and/or the impact of climate changes on PPN communities (mainly *Pratylenchus* species) and on the pattern of occurrence of ARD in Brazilian orchards in the future. In this aspect, average temperature of the wettest quarter (BIO8) can play a crucial role for ARD development.

The type of soil and altitude, unlike studies carried out in other countries (Fleming et al. 2016; Divers et al. 2019), did not influence the abundance of PPNs. This inconsistency in relation to dissimilar trend can be explained by the small variability of types of soil in the sampled locations (two soil types). In relation to PPN incidence, we can raise an important question: after all, what factors could justify the non-influence of these bioclimatic variables in the PPN distribution? Although it is a topic that needs further studies, the use of infected seedlings appears as strongly enough hypothesis.

Populations of *P. penetrans* from different geographic areas can present high levels of morphological and morphometric variation, induced by environmental variations. Despite the tail shape and crenation to be relevant for the identification of *Pratylenchus* species (Castillo and Vovlas 2007; Gonzaga 2006), its analysis alone is not sufficient for specific characterization due to the high morphological variability (Roman and Hirschmann 1969). There was a prevalence of the sub-hemispheric tail type with a smooth terminal, and, to a lesser extent, there were less rounded and more pointed tails, sometimes crenate. Frederick and Tarjan

(1989) confirm the predominance of sub-hemispherical tails for the species, but other authors state that they tend to be moderately rounded and with a short and smooth hyaline terminal despite the possibility of variations in tails (Roman and Hirschmann 1969), such as the rare, pointed tails with a crenate terminal (Gonzaga 2006). The multivariate analyses confirmed the specific identities of the OTUs studied and defined a priori, which proved to be important tools in taxonomic studies.

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Author contribution All authors contributed to the contextualization of the study. The collection of data and study logistics was performed by EKKR and PCP. Data analysis and interpretation were carried out by EKKR, LJD and JVAF. The manuscript (original draft) was written by EKKR, LJD and JVAF. All the authors approved the manuscript.

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Data availability The datasets obtained during this study are available from the corresponding author on reasonable request.

Declarations

Conflict of interest The authors declare no competing interests.

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