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Plant-parasitic nematodes associated with bush tea (*Athrixia phylicoides*) in South Africa and their relationship with physico-chemical soil properties

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Summary – Bush tea (*Athrixia phylicoides*) is an aromatic, perennial, leafy shrub that is endemic to the northeastern mountain ranges of South Africa and has a high potential for commercialisation as an alternative to caffeine-containing tea. During the summer and winter of 2018-2019, a survey was carried out at nine localities in the northeastern regions of South Africa to study the diversity of nematodes associated with bush tea and, in terms of frequency of occurrence and abundance, identify the dominant plant-parasitic nematodes. Twenty-one plant-parasitic nematode species belonging to 14 genera were identified in 90 rhizosphere soil and root samples. *Meloidogyne* and *Helicotylenchus* were the dominant plant-parasitic nematode genera. *Meloidogyne javanica* and *Scutellonema brachyurus* were found at all localities, followed by *M. enterolobii*, *Pratylenchus brachyurus*, *Rotylenchulus parvus*, *H. martini* and *S. truncatum* (found at 7-8 localities). Other species identified included *Criconema corbetii*, *C. sphaerocephalus*, *C. xenoplax*, *Criconemoides ihlathum*, *C. parvus*, *Discocriconemella glabrannulata*, *H. dihystera*, *H. erythrinae*, *H. paraplatyurus*, *Hemicycliophora typica*, *M. hapla*, *M. incognita*, *Rotylenchulus unisexus* and *R. clavicaudatus*. Individuals of *Crossonema*, *Paratylenchus*, *Ogma*, the *Xiphinema americanum*-group and *X. americanum sensu lato* could not be identified to species level due to the low number of specimens present in the samples. Comparison of the two methods used to identify the *Meloidogyne* populations to species level shows that morphological identification (particularly perineal pattern morphology) provided a more complete picture of the *Meloidogyne* species present in the samples compared with the molecular SCAR-PCR technique. High levels of Cu, K and pH were associated with the highest relative population densities (RPD% = average population density of a nematode genus/total nematode population density × 100) of *Meloidogyne*, whilst high levels of Al and soil resistivity were associated with the lowest RPD% of *Meloidogyne*. By contrast, high levels of K and pH were associated with the lowest RPD% of *Helicotylenchus*, whilst high levels of Al and soil resistivity were associated with the highest RPD% of *Helicotylenchus*.

Keywords – diversity, nematode-soil relationships, rainfall, root-knot nematodes, spiral nematodes, temperature.

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Together with rooibos (*Aspalathus linearis*) and honeybush (*Cyclopia* ssp.), bush tea (*Athrixia phylicoides*) is a plant species that has a long and rich ethnobotanical history of use by indigenous people as a medicinal tea (Joubert *et al*., 2008; Lerotholi *et al*., 2017). Bush tea is an aromatic, perennial, leafy shrub that can reach a height of up to 1 m (Fox & Young, 1982). It is endemic to the northeastern mountain ranges of Southern Africa and is well adapted to altitudes ranging from 600 to 2000 m a.s.l. (Lehlohonolo *et al*., 2013; Lerotholi *et al*., 2017). Its geographical distribution ranges from the northern parts of the Limpopo Province, throughout the Mpumalanga Province, to large areas of the eastern part of South Africa, including the KwaZulu-Natal and Eastern Cape provinces, Eswatini (formerly Swaziland) and Lesotho (Lerotholi *et al*., 2017). Bush tea grows well in a range of diverse climates and habitats, such as grasslands, bushveld, forests and rocky slopes (Lerotholi *et al*., 2017). Plant growth and yield, as well as chemical composition, are affected by climatic and abiotic and biotic soil factors (Mudau *et al*., 2007; Chabeli *et al*., 2008; Lehlohonolo *et al*., 2013). In the region of South Africa where bush tea is growing, the summer and winter seasons vary considerably in rainfall and temperature resulting in differences in plant growth. Plants flower throughout the year, although the best flowering months are from March to May.

The potential for commercialising bush tea as an alternative to caffeine-containing tea is well documented (Joubert *et al*., 2008; Lehlohonolo *et al*., 2013) but, despite the high economic potential of bush tea as a crop with high export value, the commercialisation of bush tea is still in its infancy (Lerotholi *et al*., 2017). Most research and development efforts on bush tea have focused so far on cultural practices, such as the effects of the application of macro- and micro-nutrients on plant growth (Mudau *et al*, 2005, 2007; Mogotlane *et al*., 2007), pruning and application of growth regulators (Maudu *et al*., 2010, 2011; Marasha *et al*., 2013). So far, very limited attention has been paid to the occurrence of diseases and pests, including plant-parasitic nematodes, which may have the potential to limit the cultivation of bush tea.

Recently, based on indications that honeybush is highly susceptible to root-knot nematodes (Hart *et al*., 2005), and the description of *Helicotylenchus curatus* (Marais *et al*., 2004) and the findings of the dagger nematode, *Xiphinema oxycaudatum*, and the root-lesion nematode, *Pratylenchus bolivianus*, on honeybush and rooibos, respectively (Daramola *et al*., 2018, 2019), in South Africa, an intensive survey of plant-parasitic nematodes associated with honeybush and rooibos in the Western Cape Province of South Africa was carried out (Daramola *et al*., 2021a). Twenty-six nematode families were identified from honeybush fields while 24 were identified from rooibos fields. Inspired by the results of this survey and since, to our knowledge, no information on plant-parasitic nematodes association with bush tea in South Africa is available, the main objective of our study was to determine the diversity of nematodes associated with bush tea and, in terms of frequency of occurrence and abundance, identify the dominant plant-parasitic nematodes. Since using only morphological characteristics (particularly perineal pattern morphology) is deemed problematic for the identification of *Meloidogyne* species (Visagie *et al*., 2018), the *Meloidogyne* populations found in our study were identified using both morphological characteristics and the SCAR-PCR technique, which has been proven to be a reliable method to discriminate *Meloidogyne* species (Zijlstra *et al*., 2000). Since the community structure of nematodes can be affected by a wide variety of climatic and abiotic factors, such as temperature, rainfall, soil texture, pH, *etc*. (Song *et al*., 2017; Liao *et al*., 2021), another objective of our study was to investigate the effect of temperature and rainfall, and a selection of physico-chemical soil properties on the frequency of occurrence and abundance of plant-parasitic nematodes on bush tea. To examine climatic effects, samples were collected during both summer and winter.

Materials and methods

Localities sampled, rhizosphere soil and root sampling

Bush tea rhizosphere soil and root samples were collected during summer (December 2018-January 2019) and winter (June-July 2019) from bush tea plants growing in their natural habitat at nine localities in the northeastern region of South Africa (Fig. 1; Table 1). At each locality, ten bush tea plants, growing 4-7 m apart from each other, were, following a zig-zag pattern, uprooted using a garden fork. The above-ground parts of each plant were cut at the base using a pruning shear, the root system and rhizosphere soil placed in labelled plastic bags stored in cool boxes, which were transported to the laboratory in Mbombela. In the laboratory, the plastic bags were stored in a cold room at an ambient temperature of 5-10°C until processing of the samples.

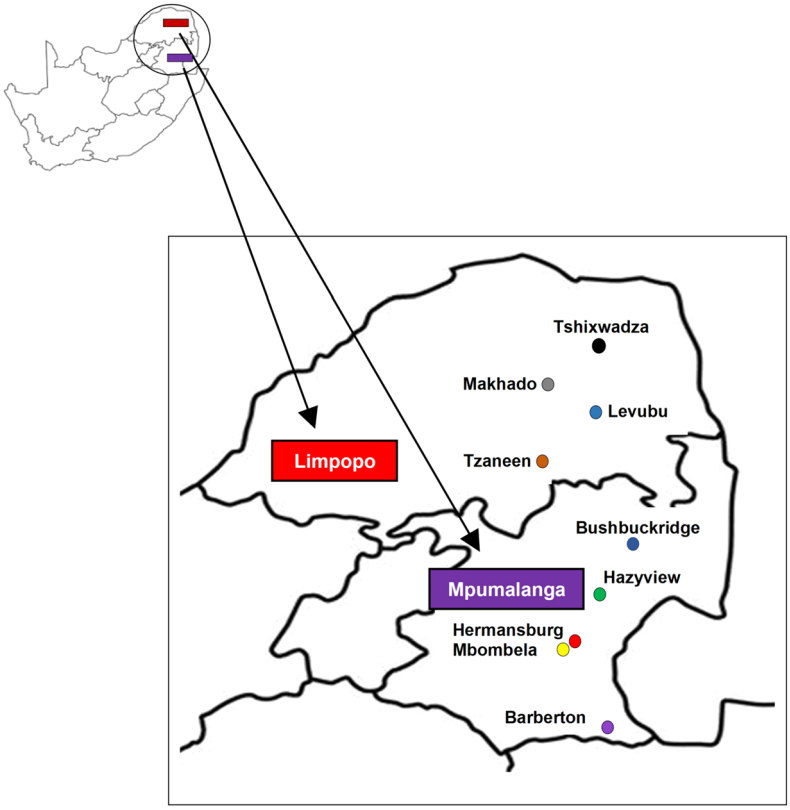

Fig. 1. Map of South Africa and the Limpopo and Mpumalanga provinces, showing the nine localities where rhizosphere soil and root samples of bush tea plants were collected.

Table 1. Agro-ecological zone, altitude, average minimum and maximum temperature, average annual rainfall, and soil texture and classification of the nine localities sampled.

Locality	A-E Zone	Altitude (m)	Min. temp. (°C)	Max. temp. (°C)	Annual rainfall (mm)	pH	Sand (%)	Loam (%)	Clay (%)	Soil type
Barberton	Cwa	877	14.0	28.4	57.3	6.9	88.6	2.0	9.4	Sand
Bushbuckridge	Cwa	858	16.0	29.7	42.9	5.5	80.0	5.4	14.6	Loamy sand
Hazyview	Cwa	857	13.9	28.8	54.5	6.4	79.5	4.5	16.0	Loamy sand
Hermansburg	Cwa	676	14.0	29.4	59.9	5.8	82.6	3.8	13.6	Loamy sand
Levubu	Bsh	880	15.5	28.0	100.5	5.8	50.8	15.2	34.0	Sandy clay
Makhado	Bsh	935	13.6	28.1	64.6	6.0	53.8	14.0	32.2	Sandy clay loam
Mbombela	Cwa	680	14.0	29.4	59.9	6.1	89.1	2.6	8.3	Sand
Tshixwadza	Bsh	615	13.6	28.1	64.6	5.4	68.0	5.2	26.8	Sandy clay loam
Tzaneen	Cwa	1402	15.3	27.2	62.9	5.4	67.1	7.0	25.9	Sandy clay loam

A-E Zone: agro-ecological zone: humid subtropical climate (Cwa); hot semi-arid climate (BSh) (Adeola *et al.*, 2017).

PROCESSING OF SAMPLES

The rhizosphere soil was carefully separated from the root system and the rhizosphere soil and roots were processed separately. The rhizosphere soil was thoroughly mixed and the nematodes extracted from one sub-sample of 250 ml soil by a modified decanting and sieving method, using stacked 150-, 45-, 38- and 25-μm-aperture

sieves, followed by the sugar centrifugal-flotation method (Marais *et al*., 2017). Soil and organic material adhering to the root systems were carefully removed by gently washing the roots with running tap water. The root system was weighed, the roots cut into fragments of 2 to 5 cm, thoroughly mixed and the nematodes extracted from one sub-sample of 10 g roots. The root fragments were first macerated in 250 ml water for 30 s using a kitchen blender at high speed and then the homogenate decanted through stacked 300-, 150-, 38- and 25-μm-aperture sieves. The residue retained on the 300-μm-aperture sieve was discarded; the residues retained on the other sieves were rinsed into a 250 ml beaker and the nematodes extracted by the sugar centrifugal-flotation method (Marais *et al*., 2017). After centrifugation, the supernatant containing either the rhizosphere soil or the root nematodes, was decanted through a 25-μm-aperture sieve, rinsed with distilled water, and washed with distilled water into a 100 ml plastic bottle, which was stored at 5-10°C until identification and counting of the nematodes.

Nematode identification and counting

The extracted nematodes (both free-living and plant-parasitic) were identified to genus level using morphological characteristics of the second-stage juveniles (J2) and adults, such as the shape of the head and tail region, stylet, *etc*. (Hunt *et al*., 2005) using a dissecting microscope (Wild Heerbrugg). Nematodes were counted in two 1 ml sub-samples using a Peter's counting slide and expressed as the number of nematodes in 250 ml rhizosphere soil or 10 g roots. After identification to genus level and counting, the nematodes were killed and fixed in a hot (80°C) fixative containing 10 ml of a 40% formaldehyde solution and 2 ml glycerol made up to 100 ml with distilled water, transferred to anhydrous glycerin and mounted on permanent slides by the paraffin wax-ring method (Marais *et al*., 2017). The slides were then sent to ARC-Plant Health and Protection (ARC-PHP), Nematology Unit, Biosystematics, Roodeplaat, South Africa, for identification of the nematodes to species level.

Morphological characterisation of root-knot nematodes

For each locality, the rhizosphere soil remaining after nematode extraction and soil analysis was thoroughly mixed and put into 5 l plastic pots. In these pots, two 6-week-old seedlings of tomato (*Lycopersicum esculentum* L.) 'Monica' (a susceptible cultivar) were planted to rear the root-knot nematodes present as either eggs or J2 in the rhizosphere soil. The pots were placed in a glasshouse at an ambient temperature of 12-32°C. Twelve weeks after transplanting of the seedlings, the tomato plants were carefully removed from the soil and the root systems gently washed with running tap water to remove soil and organic material adhering to the roots. Using a dissecting microscope, roots with galls were randomly selected and the galls dissected to collect 30 mature, egg-laying females (ELF) that were carefully removed using a fine needle and transferred to a drop of 100% lactic acid on a flat glass surface until permanent mounting of the perineal patterns according to the method described by Marais *et al*. (2017). Morphological identification of the *Meloidogyne* populations to species level was based on the morphology of the perineal region (Yang & Eisenback, 1983; Kleynhans, 1991; Karssen *et al*., 2013; Rashidifard *et al*., 2019a). After initial identification of the females at ARC-TSC, the slides were sent to ARC-PHP for confirmation of the *Meloidogyne* species identification.

Molecular identification of root-knot nematodes

Thirty living mature ELF were removed from root galls following the procedures described above and transferred to Eppendorf tubes containing 20 μl double-distilled (dd; Milli-Q) H_2O and the tubes stored at -10°C until DNA extraction from individual females using the Chelex-100 protocol of Musapa *et al*. (2013). Chelex (5%, w/v; 20 μl) and 3 μl proteinase K (20 mg ml^{-1}) were added to each Eppendorf tube and the tubes incubated at 56°C for 2 h and at 95°C for 10 min before they were stored at -20°C. Amplification of DNA was carried out using a Vacutec thermocycler (www.vacutec.co.za). The amplification reaction consisted of 25 μl of a PCR mix that was made up by adding 12.5 μl ready-to-use master mix (Promega), 1 μl forward primer (10 μM), 1 μl reverse primer (10 μM), 5 μl DNA and 5.5 μl ddH_2O. The programmes used during the PCR process for each primer included initial denaturation for 3 min at 94°C, 35 cycles of denaturation for 45 s at 94°C, annealing for 45 s at the specific temperatures required for each *Meloidogyne* species, extension for 1 min at 72°C and, finally, an extension cycle of 6 min at 72°C followed by a holding temperature of 4°C. Annealing temperatures were 54°C for the Finc/Rinc primers; 60°C for the Fh/Rh primers; 64°C for the Fjav/Rjav and Fme/Rme primers (Zijlstra *et al*., 2000; Long *et al*., 2006). The primer sequences used are

Table 2. Codes of primers used for the molecular identification of the *Meloidogyne* populations isolated from bush tea, their sequence and DNA fragment size.

Primer code	Primer sequence (5′ → 3′)	DNA fragment size (bp)	Reference
F*Me	AAC TTT TGT GAA AGT GCC GCT G	250	Long *et al*., 2006
R*Me	TCA GTT CAG GCA GGA TCA ACC		
Finc	CTC TGC CCA ATG AGC TGT CC	1200	Zijlstra *et al*., 2000
Rinc	CTC TGC CCT CAC ATT AGG		
Fjav	GGT GCG CGA TTG AAC TGA GC	700	Zijlstra *et al*., 2000
Rjav	CAG GCC CTT CAG TGG AAC TAT AC		
Fh	TGA CGG CGG TGA GTG CGA	610	Zijlstra *et al*., 2000
Rh	TGA CGG CGG TAC CTC ATA G		

*F: forward; R: reverse.

Table 3. *Meloidogyne* populations used as positive controls for the molecular identification of the *Meloidogyne* populations isolated from bush tea.

Meloidogyne species (mature females)	Province of origin; host plant	Code	Reference
M. javanica	Limpopo; tomato	P28	Rashidifard *et al*. (2019b)
M. incognita	Limpopo; tomato	P34	Rashidifard *et al*. (2019b)
M. enterolobii	Mpumalanga; guava	P1	Rashidifard *et al*. (2019b)
M. hapla	Mpumalanga; potato	P9	Visagie *et al.*, (2018)

listed in Table 2. The South African populations of *Meloidogyne enterolobii* (P1), *M. incognita* (P34), *M. javanica* (P28) and *M. hapla* (P9), identified by Rashidifard *et al*. (2019b) and Visagie *et al*. (2018), were used as positive controls for the respective *Meloidogyne* species identified during the SCAR-PCR processes (Table 3). After completion of the DNA amplification, 4 μl of PCR product of each sample, representing the nine *Meloidogyne* populations, were loaded on a 1% agarose gel to determine the quality of the DNA products. The DNA bands were stained with GelRed (www.biotium.com) and visualised and photographed using an UV transilluminator (Chemi-Doc MP Imaging; Biorad). The remaining products of each sample were stored at −20°C prior to sequencing by the genomic company Inqaba Biotec™, Pretoria, South Africa (www.inqaba-southafrica.co.za). Primers used for the sequencing reactions were the same as those used in the SCAR-PCR process.

Physico-chemical soil properties

From each rhizosphere soil sample, 500 g soil was sent to the soil analytical laboratory of ARC-TSC to analyse the percentages C, sand, loam, clay and organic matter, pH (KCl; 1:2.5), P (Bray 1; mg kg^{-1}), Na (mg kg^{-1}), Mg (mg kg^{-1}), K (mg kg^{-1}), Ca (mg kg^{-1}), N-NO3 (mg kg^{-1}), N-NH4 (mg kg^{-1}), Al (mg kg^{-1}) and soil resistivity (Ohm) using standard protocols (HSST, 1990).

Climatic data

Climatic data (average minimum and maximum temperatures; annual rainfall) of the last 5 years were obtained from Agricultural Research Council-Soil, Climate and Water (ARC-SCW), Pretoria. The average of these 5 years was used for the analyses (Table 1).

Statistical analysis

For each genus and species identified, the mean population density (MPD), frequency of occurrence (F%) and prominence value (PV) were calculated using the following equations (De Waele & Jordaan, 1988): MPD = total number of individuals/number of samples; F% = number of samples in which either the genus or species occurred/total number of samples × 100; PV = population density of either each genus or species × square root of the frequency of occurrence/number of samples.

Multivariate analyses were used to investigate the relationships between the plant-parasitic nematode populations (response variables) and the environmental data measured (explanatory variables) using co-inertia analysis. Datasets used were the relative population densities

(RPD%) of the plant-parasitic nematode genera at each locality (RPD% = average population density of a nematode genus/total nematode population density × 100; Nisa *et al*., 2021), and soil physico-chemical properties and climatic data. Monte Carlo permutation tests were used to determine the significance of the effect of the explanatory variables on the response variables. Biplots were created using ADE-4 Software (Thioulouse *et al*., 1997). The relationships were then analysed using ANOVA to determine significant differences between the RPD% of the plant-parasitic nematode genera and physico-chemical soil properties with the highest factorial values using a *t*-test analysis. The RPD% of *Meloidogyne* and *Helicotylenchus* were sorted according to the 20 highest and 20 lowest values of the physico-chemical soil properties and significance according to $P \leqslant 0.05$ was determined.

Results

A total of 90 rhizosphere soil and 90 root samples were collected. In these samples, 21 plant-parasitic nematode species belonging to 14 genera were identified to species level (Table 4). The populations of the genera *Crossonema* and *Ogma* (both found at only one locality) and *Paratylenchus* (found at five localities), *Discocriconemella* (one population) and *Pratylenchus* (one population), *Rotylenchus* (six populations) and *Rotylenchulus* (eight populations) could not be identified to species level because only juveniles were found in those samples. Populations of species belonging to the *Xiphinema americanum*-group and *X. americanum sensu lato* were found at five and one locality, respectively. *Meloidogyne javanica* and *Scutellonema brachyurus* were found at all localities, followed by *M. enterolobii*, *Pratylenchus brachyurus* and *Rotylenchulus parvus* (all three found at eight localities) and *Helicotylenchus martini* and *Scutellonema truncatum* (both found at seven localities). In addition to the genera *Crossonema* and *Ogma*, seven species, and the *X. americanum sensu lato* population were found at only one locality.

In the rhizosphere soil, nine plant-parasitic nematode genera were found in addition to Criconematidae. In terms of frequency of occurrence and population density, *Helicotylenchus* and *Meloidogyne* were the most frequent and abundant nematode genera found (data not shown). *Helicotylenchus* was found at all localities, in 92 and 86% of the rhizosphere soil samples during summer (MPD = 893) and winter (MPD = 1164), respectively; *Meloidogyne* was also found at all localities, in 89 and 97% of the rhizosphere soil samples during summer (MPD = 645) and winter (MPD = 1302), respectively. Criconematidae were found at all localities, in 56 and 40%

Table 4. Plant-parasitic nematode taxa isolated from rhizosphere soil and roots of bush tea at nine localities in the Limpopo and Mpumalanga provinces of South Africa.

Nematode taxa	Ba	Bu	Ha	He	Le	Ma	Mb	Ts	Tz
Criconema corbetii	–	X	X	X	–	X	–	–	–
Mesocriconema sphaerocephalum	–	–	–	–	–	–	–	–	X
Mesocriconema xenoplax	–	–	–	–	X	–	–	X	–
Criconemoides ihlathum	–	–	–	–	X	–	–	–	–
Criconemoides parvus	–	–	X	X	–	X	X	–	–
Crossonema spp.	–	–	–	–	–	X	–	–	–
Discocriconemella glabrannulata	X	X	X	X	–	–	X	–	X
Discocriconemella spp.	–	–	–	–	–	X	–	–	–
Helicotylenchus dihystera	–	–	–	–	–	–	–	–	X
Helicotylenchus erythrinae	–	–	–	–	–	–	–	–	X
Helicotylenchus martini	X	X	–	X	X	X	X	X	–
Helicotylenchus paraplatyurus	–	–	X	–	–	–	–	–	–
Hemicycliophora typica	–	–	–	–	–	–	X	–	–
Meloidogyne enterolobii	X	X	X	X	X	X	–	X	X
Meloidogyne hapla	–	–	–	–	–	X	–	–	–
Meloidogyne incognita	X	X	X	–	X	X	–	–	X
Meloidogyne javanica	X	X	X	X	X	X	X	X	X
Pratylenchus brachyurus	X	X	X	X	–	X	X	X	X
Pratylenchus spp.	–	–	–	–	X	–	–	–	–
Paratylenchus spp.	X	–	–	–	X	X	X	X	–
Rotylenchus unisexus	–	–	–	X	–	X	–	X	–
Rotylenchus spp.	X	X	X	–	X	–	X	–	X
Rotylenchulus clavicaudatus	–	–	–	–	–	X	–	–	–
Rotylenchulus parvus	X	X	X	X	–	X	X	X	X
Rotylenchulus spp.	X	X	–	X	X	X	X	X	X
Scutellonema brachyurus	X	X	X	X	X	X	X	X	X
Scutellonema truncatum	–	X	X	X	X	X	X	X	–
Ogma spp.	–	–	–	–	–	–	–	X	–
Xiphinema americanum-group	–	–	X	X	–	–	X	X	X
Xiphinema americanum sensu lato	–	–	–	–	–	–	–	X	–

Ba: Barberton; Bu: Bushbuckridge; Ha: Hazyview; He: Hermansburg; Le: Levubu; Ma: Makhado; Mb: Mbombela; Ts: Tshixwadza; Tz: Tzaneen. X: present; -: absent.

of the rhizosphere soil samples during summer and winter, respectively, but in low population densities (MPD = 109 and 106 during summer and winter, respectively). *Hemicycliophora* was only found during winter at one locality, in 7% of the rhizosphere soil samples, and in low population densities (MPD = 38). *Paratylenchus*, *Pratylenchus* and *Xiphinema* occurred at only a few localities, in general in < 10% of the rhizosphere soil samples, and in low population densities (MPD < 50) during both summer and winter. *Rotylenchulus* was found at all localities during winter (22% of the rhizosphere soil samples; MPD = 84) but only at five localities during summer (21% of rhizosphere soil samples; MPD = 48). In general, the nematode population densities were higher in winter compared with summer. For example, population densities of *Helicotylenchus* and *Meloidogyne* were on average 30 and 102% higher in winter compared with summer, respectively.

In the roots, four plant-parasitic nematode genera were found: *Meloidogyne*, *Helicotylenchus*, *Pratylenchus* and *Rotylenchulus*. In terms of frequency of occurrence and population density, *Meloidogyne* was the most frequent and abundant genus followed by *Helicotylenchus* (data not shown). *Meloidogyne* was found at all localities, in 99 and 81% of the root samples during summer (MPD = 765) and winter (MPD = 306), respectively; *Helicotylenchus* was found at eight and seven localities, in 22 and 42% of the root samples during summer (MPD = 25) and winter (MPD = 47), respectively. *Pratylenchus* was found at all localities during summer, in 22% of the root samples, but at only five localities, in 12% of the root samples, during winter and during both seasons in low population densities (MPD < 5). *Rotylenchulus* was also found during both summer and winter but at only two and three localities, in < 6% of the root samples, and in low population densities (MPD = 4 and 13), respectively.

With regard to the localities sampled, the number of nematode species found in the rhizosphere soil and roots ranged from eight to 13 (Table 4). In the rhizosphere soil, population densities of *Meloidogyne* > 1000 (250 ml soil)$^{-1}$ were found at one locality during summer *vs* three localities during winter (Table 5). By contrast, population densities of *Helicotylenchus* > 1000 (250 ml soil)$^{-1}$ were found at four and five localities during summer and winter, respectively. Four localities had population densities of *Helicotylenchus* > 1000 (250 ml soil)$^{-1}$ during both seasons. At five localities, population densities of *Helicotylenchus* were higher during summer compared with winter and *vice versa* at four localities. For the other genera found in the rhizosphere soil, population densities were less than 500 (250 ml soil)$^{-1}$ (with one exception) and no localities with significantly higher population densities compared with the other localities were observed.

In the roots, population densities of *Meloidogyne* > 1000 (10 g roots)$^{-1}$ were found at two localities during summer and at one locality during winter (Table 6). At the other localities, population densities of *Meloidogyne* ranged from 416 to 811 (10 g roots)$^{-1}$ during summer *vs* 84 to 385 (10 g roots)$^{-1}$ during winter. In general, population densities of *Helicotylenchus* were < 100 (10 g roots)$^{-1}$ (with one exception) and those of *Pratylenchus* < 50 (10 g roots)$^{-1}$. At three localities, population densities of *Helicotylenchus* were higher during summer compared with winter and *vice versa* at six localities. *Pratylenchus* was found in the roots at all localities during summer but in only three localities during winter.

Variability in the morphology of the perineal pattern was observed within and between *Meloidogyne* populations. The perineal pattern in *M. enterolobii* was generally round- to oval-shaped with a moderately high dorsal arch and distinct phasmids on the tail terminus; in *M. javanica*, the perineal pattern was mostly circular- to oval-shaped with a low to medium dorsal arch, the apex of the dorsal arch squarish to broadly rounded, inner striae above tail terminus low to high, lateral lines distinct; in *M. incognita*, the perineal pattern was mostly circular- to oval-shaped with a medium high to high dorsal arch, the apex of the dorsal arch squarish or broadly rounded, the junction between the lateral lines y-shaped; in *M. hapla*, the perineal pattern was circular to oval-shaped with a low to medium dorsal arch and the apex of the dorsal arch broadly rounded, the inner striae above the tail terminus low and round.

SCAR-PCR analysis of DNA of *M. enterolobii*, *M. javanica* and *M. incognita* resulted in the 200, 700 and 1200 bp SCAR fragments, respectively, being amplified using the *M. enterolobii*, *M. javanica* and *M. incognita* species-specific primers, respectively (Fig. 2). DNA fragments that were amplified for the respective *Meloidogyne* species, namely 200 bp for *M. enterolobii*, 700 bp for *M. javanica* and 1200 bp for *M. incognita*, corresponded with the lengths of the DNA fragments obtained for the positive standards for each of the three *Meloidogyne* species. By contrast, the 610 bp amplified DNA fragment did not correspond with the length of the DNA fragment obtained for the positive standard of *M. hapla*.

Table 5. Mean population densities (MPD) of the major nematode genera recovered from 250 ml rhizosphere soil of bush tea during either summer or winter at nine localities in South Africa.

Locality	*Meloidogyne*	*Helicotylenchus*	*Scutellonema*	*Rotylenchus*	*Pratylenchus*	*Criconemella*	*Rotylenchulus*	*Paratylenchus*	*Xiphinema*	*Hemicycliophora*
Summer										
Barberton	985 ± 469*	335 ± 481	95 ± 138	130 ± 146	25 ± 49	110 ± 154	8215 ± 42	0	0	0
Bushbuckridge	500 ± 304	510 ± 360	290 ± 609	50 ± 78	0	235 ± 231	40 ± 88	0	0	0
Hazyview	630 ± 1020	945 ± 891	90 ± 149	105 ± 183	10 ± 21	115 ± 88	115 ± 141	0	25 ± 54	0
Hermansburg	615 ± 370	1290 ± 738	0	0	15 ± 34	190 ± 178	0	0	30 ± 54	0
Levubu	1055 ± 661	110 ± 158	5 ± 16	10 ± 21	0	10 ± 21	0	5 ± 16	0	0
Makhado	515 ± 708	1225 ± 1015	140 ± 210	120 ± 134	0	55 ± 96	10 ± 21	5 ± 16	0	0
Mbombela	595 ± 834	1240 ± 1206	45 ± 76	265 ± 231	5 ± 16	70 ± 118	5 ± 16	0	90 ± 137	340 ± 435
Tshixwadza	520 ± 464	995 ± 766	295 ± 482	100 ± 108	5 ± 16	15 ± 34	50 ± 111	25 ± 49	0	0
Tzaneen	39 0 ± 493	1385 ± 1658	135 ± 256	125 ± 183	20 ± 35	180 ± 318	0	0	49 ± 75	0
Winter										
Barberton	300 ± 224	70 ± 92	0	30 ± 54	0	70 ± 63	55 ± 114	5 ± 16	0	0
Bushbuckridge	635 ± 543	230 ± 211	10 ± 32	5 ± 16	0	455 ± 559	85 ± 136	0	0	0
Hazyview	160 ± 104	185 ± 246	30 ± 48	50 ± 108	5 ± 16	35 ± 94	25 ± 49	0	5 ± 16	0
Hermansburg	2785 ± 1464	3265 ± 4359	10 ± 21	35 ± 58	0	170 ± 284	525 ± 1147	0	50 ± 125	0
Levubu	1585 ± 2488	730 ± 1257	30 ± 63	30 ± 95	5 ± 16	5 ± 16	20 ± 63	25 ± 79	0	0
Makhado	3185 ± 3483	1325 ± 1297	160 ± 263	90 ± 185	0	5 ± 16	15 ± 34	230 ± 627	0	0
Mbombela	860 ± 610	1080 ± 1154	70 ± 118	15 ± 24	0	35 ± 111	10 ± 32	15 ± 47	0	0
Tshixwadza	550 ± 668	2640 ± 1675	165 ± 170	115 ± 82	10 ± 21	40 ± 61	20 ± 35	5 ± 16	20 ± 48	0
Tzaneen	420 ± 451	2195 ± 2120	150 ± 219	75 ± 138	55 ± 101	135 ± 147	0	0	35 ± 94	0

*Mean value followed by standard deviation (SD).

Table 6. Mean population densities (MPD) of the major nematode genera recovered from 10 g roots of bush tea during either summer or winter at nine localities in South Africa.

Locality	Winter				Summer			
	Meloidogyne	*Pratylenchus*	*Helicotylenchus*	*Rotylenchulus*	*Meloidogyne*	*Pratylenchus*	*Helicotylenchus*	*Rotylenchulus*
Barberton	103 ± 137	11 ± 25	0	6 ± 17	1344 ± 722	39 ± 63	70 ± 80	0
Bushbuckridge	85 ± 104	0	13 ± 22	4 ± 9	814 ± 857	3 ± 9	0	0
Hazyview	95 ± 97	9 ± 19	21 ± 50	0	506 ± 217	18 ± 39	21 ± 44	15 ± 27
Hermansburg	395 ± 405	0	176 ± 247	0	482 ± 527	26 ± 48	22 ± 48	0
Levubu	251 ± 259	0	0	0	415 ± 286	8 ± 24	5 ± 16	0
Makhado	1542 ± 1964	4 ± 12	76 ± 81	5 ± 1 6	569 ± 658	28 ± 46	11 ± 24	18 ± 39
Nelspruit	109 ± 140	0	29 ± 55	0	607 ± 452	19 ± 31	6 ± 19	0
Tshixwadza	93 ± 46	73 ± 154	52 ± 51	0	1429 ± 1337	11 ± 24	71 ± 153	0
Tzaneen	54 ± 110	20 ± 52	54 ± 85	0	717 ± 738	14 ± 44	14 ± 44	0

*Mean value followed by standard deviation (SD).

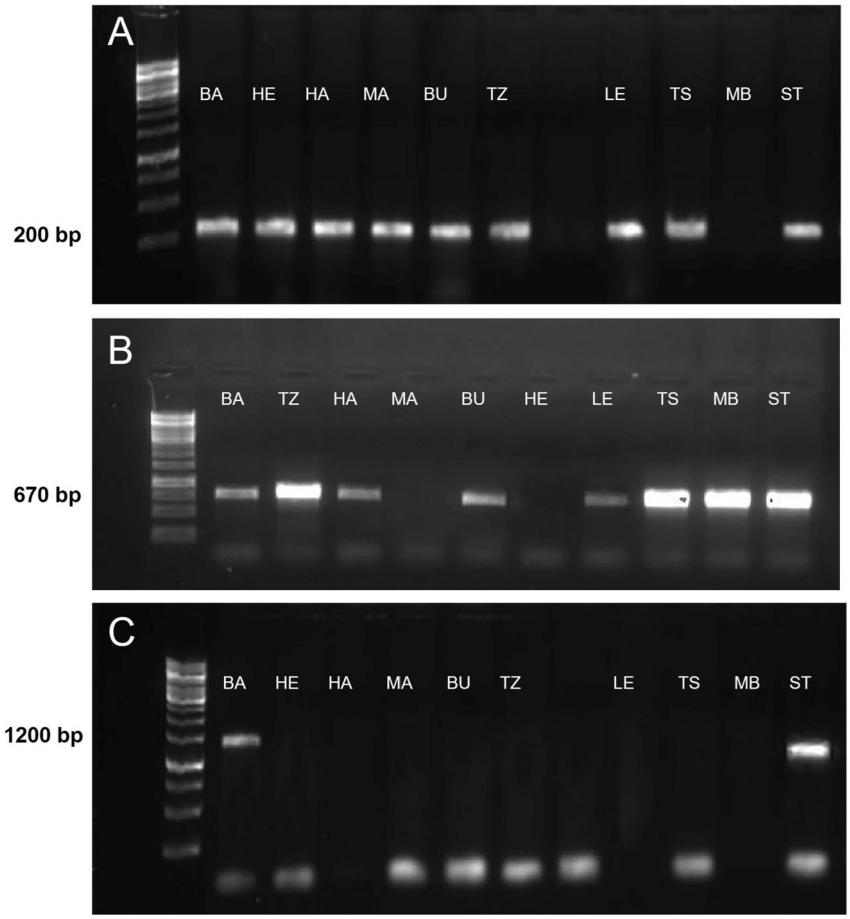

Fig. 2. Gel images of DNA fragment amplification products of females of the *Meloidogyne* populations isolated from the parasitised roots and rhizosphere soil sampled from bush tea at nine localities in South Africa using species-specific sequence characterised amplified region primers. A: *Meloidogyne enterolobii*; B: *Meloidogyne javanica*; C: *Meloidogyne incognita*. 1 kb DNA ladder (1st lane of each gel) were used for all samples; ST = DNA of standard (control) population used for each species.

Table 7. Comparison of the morphological and molecular identification of the *Meloidogyne* populations isolated from the roots of bush tea at nine localities in South Africa.

Province	Locality	Morphological identification	Molecular identification	Comparison of methods
Mpumalanga	Barberton	*M. javanica* 100%	*M. javanica* *M. incognita* *M. enterolobii*	Different
	Bushbuckridge	*M. javanica* 89% *M. incognita* 7% *M. enterolobii* 4%	*M. javanica* *M. enterolobii*	Different
	Hazyview	*M. javanica* 62% *M. incognita* 15% *M. enterolobii* 23%	*M. javanica* *M. enterolobii*	Different
	Hermansburg	*M. javanica* 50% *M. enterolobii* 50%	*M. enterolobii*	Different
	Mbombela	*M. javanica* 100%	*M. javanica*	Similar
Limpopo	Levubu	*M. javanica* 40% *M. incognita* 10% *M. enterolobii* 40% *M. hapla* 10%	*M. javanica* *M. enterolobii*	Different
	Makhado	*M. javanica* 92% *M. incognita* 8%	*M. enterolobii*	Different
	Tshixwadza	*M. javanica* 67% *M. enterolobii* 33%	*M. javanica* *M. enterolobii*	Similar
	Tzaneen	*M. javanica* 70% *M. incognita* 4% *M. enterolobii* 26%	*M. javanica* *M. enterolobii*	Different

Using morphological identification, *M. javanica* was the most dominant species found at all localities *vs* seven localities using molecular identification (Table 7). Using morphological identification, *M. enterolobii* and *M. incognita* were both found at five localities *vs* one and eight localities, respectively, using molecular identification. At Levubu, morphological identification indicated the presence of *M. hapla* but this could not be confirmed by molecular identification. At Barberton, morphological identification indicated the presence of only one *Meloidogyne* species (*M. javanica*) but molecular identification also indicated the presence of *M. enterolobii* and *M. incognita*. At Makhado, molecular identification indicated the presence of only *M. enterolobii* but morphological identification indicated the presence of *M. javanica* and *M. incognita*, not *M. enterolobii*.

Soil texture (% sand, loam and clay) differed greatly among the localities ranging from sandy to sandy clay (Table 1). The highest average *Meloidogyne* population density was found in Makhado, in a sandy clay loam soil (53.8% sand). The lowest average *Meloidogyne* population densities were found in Tzaneen and Tshixwadza, in a sandy clay loam soil (67-68% sand). The lowest average annual rainfall was 42.9 mm; the highest average annual rainfall 100.5 mm. At all other localities, average annual rainfall ranged from about 55 to 65 mm.

Eigenvalues of the multivariate analyses showed that first (F1) and second (F2) factors accounted for 26.04% of the total inertia (Fig. 3). In the correlation circle, *Meloidogyne* opposes *Helicotylenchus* having the highest positive and negative co-ordinates, respectively (Fig. 3A). Localities with relatively high RPD% of *Meloidogyne* were located on the positive side of the factorial map whereas localities with relatively low RPD% of *Meloidogyne* were located on the negative side of the factorial map (Fig. 3B) and *vice versa* for *Helicotylenchus*.

The Monte Carlo test (the permutations provided no inertia value higher than the ones obtained with the original non-permutated data) indicated that the RPD% of the plant-parasitic nematode genera and physico-chemical soil properties were correlated as well as RPD% and climatic data.

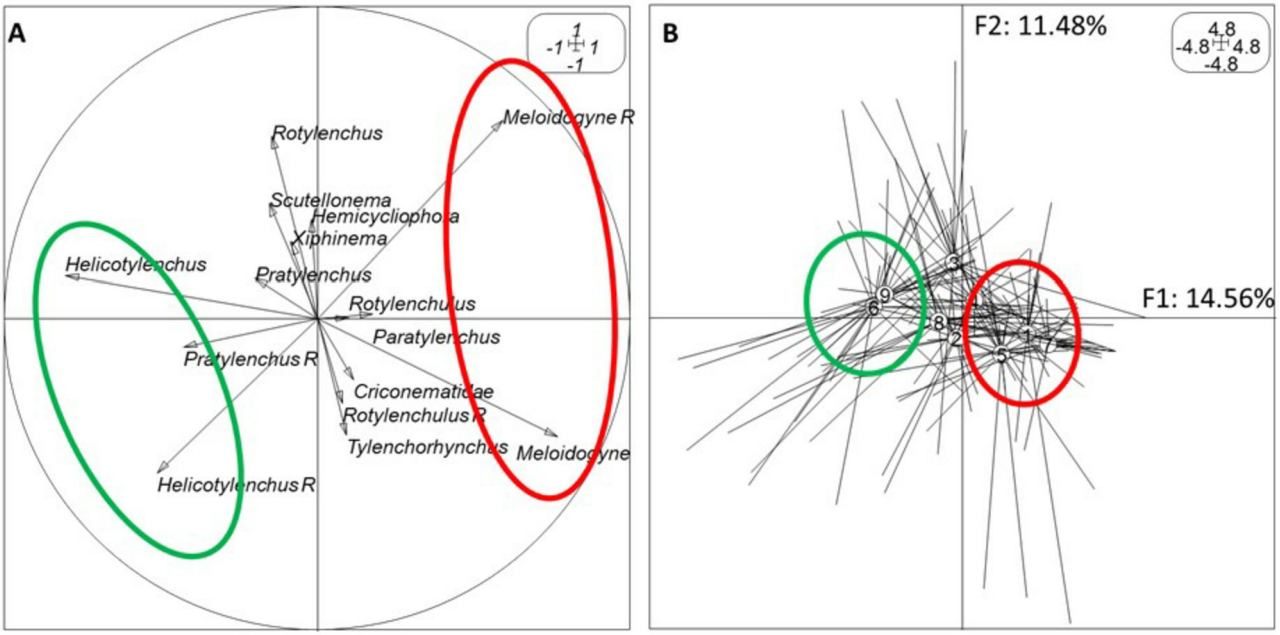

Fig. 3. Results of multivariate analyses in ADE-4. A: F1-F2 correlation circle of the nematode taxa. R indicates nematode populations isolated from the root samples; B: Factor map of the data points grouped per locality. The green circle (plotted *ad hoc*) indicates the localities positively correlated with high *Helicotylenchus* population percentages and low *Meloidogyne* population percentages, while the red circle (plotted *ad hoc*) indicates localities positively correlated with high *Meloidogyne* population percentages. 1 = Barberton, 2 = Hermansburg, 3 = Mbombela, 4 = Hazyview, 5 = Bushbuckridge, 6 = Tzaneen, 7 = Levubu, 8 = Machado and 9 = Tshixwadza. The nematode data set consists of the population density of each nematode taxon expressed as a percentage of the total nematode population density.

The factorial map of the plant-parasitic nematode populations indicated that the RPD% of *Meloidogyne* in the rhizosphere soil were strongly correlated with the positive values of F2 while situated on the positive side of F1, and that the RPD% of *Helicotylenchus* in the rhizosphere soil were strongly correlated with the negative values of F1 and F2 (Fig. 4A).

The factorial map of the soil physico-chemical properties indicated that Cu, K and pH, Ca and Ca+Mg/K, were strongly positively correlated with the positive value of F2 while positioned on the positive side of F1 and that Al and soil resistivity were strongly correlated with the negative values of F1 and F2 (Fig. 4B). High levels of Cu, K and pH were associated with the highest RPD% of *Meloidogyne* while high levels of Al and soil resistivity were associated with the lowest RPD% of *Meloidogyne*.

The physico-chemical soil properties with the highest positive and negative correlations with the RPD% of *Meloidogyne* in the rhizosphere soil were sorted from high to low values after which the averages of the 20 highest and 20 lowest values were calculated and compared with the averages of the RPD% of *Meloidogyne* and *Helicotylenchus* for these samples. Using the *t*-test, significant differences between *Meloidogyne* and *Helicotylenchus* for these selected physico-chemical soil properties were observed, with the exception of *Helicotylenchus* and Cu (Table 8). Co-inertia analysis of either the summer or winter climatic data sets separately showed that the same physico-chemical soil properties (Cu, K, pH, Al and soil resistivity) were positively and/or negatively correlated with the RPD% of *Meloidogyne* and *Helicotylenchus* (data not shown), indicating that the results were independent of the season.

Co-inertia analysis showed that high RPD% of *Meloidogyne* in the rhizosphere soil were positively correlated with high minimum temperatures and high annual rainfall (data not shown). High RPD% of *Helicotylenchus* were negatively correlated with all climatic data included in our study.

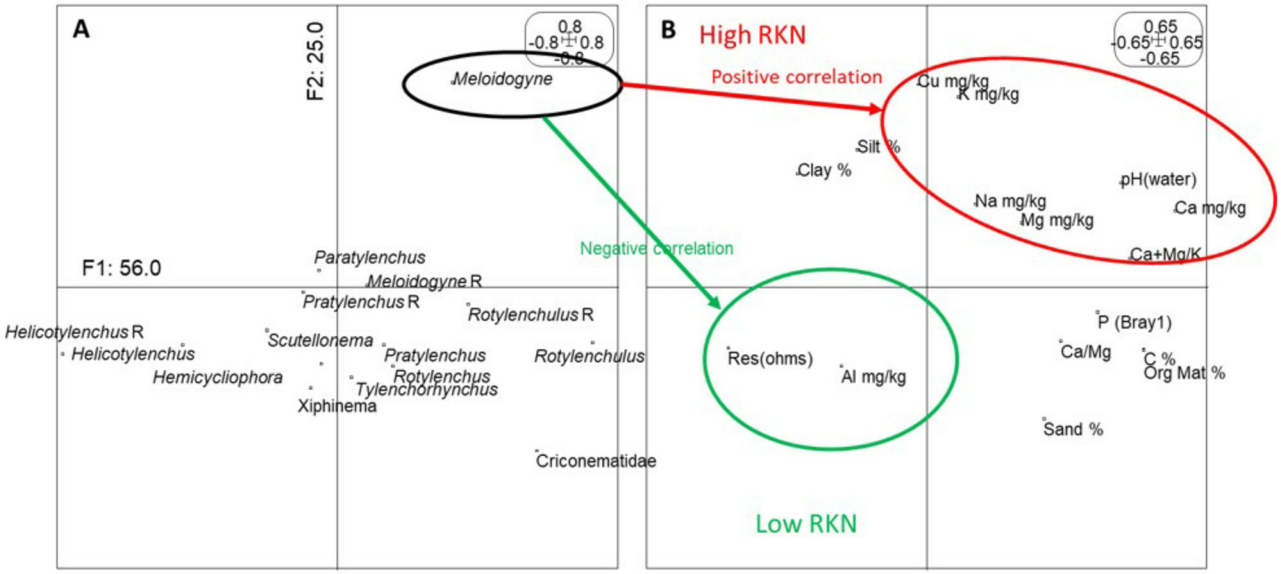

Fig. 4. Results of co-inertia analysis in ADE-4. Factor maps of the nematode taxa (A) and physico-chemical soil properties (B) after co-inertia analysis. R indicates nematode populations isolated from the root samples. The green circle (plotted *ad hoc*) indicates the physico-chemical soil properties negatively correlated with high *Meloidogyne* population percentages (low RKN), while the red circle (plotted *ad hoc*) indicates physico-chemical soil variables positively correlated with high *Meloidogyne* population percentages (high RKN). The nematode data set consists of the population density of each nematode taxon expressed as a percentage of the total nematode population density.

Table 8. Relationships between the relative population densities (RPD% = average population density of a nematode genus/total nematode population density × 100) of *Meloidogyne* and *Helicotylenchus* extracted from the rhizosphere soil in the 20 samples with the highest and lowest values of Cu, K and Al, pH and soil resistivity.

	High	SE	Low	SE	*t*-test
Cu (mg kg^{-1})	15.75	0.699	0.24	0.016	***
Meloidogyne	60.1	8.24	31.1	5.71	*
Helicotylenchus	31.7	8.07	44.3	5.41	ns
K (mg kg^{-1})	218.8	10.32	37.3	0.616	***
Meloidogyne	64.6	6.75	25.6	5.57	***
Helicotylenchus	29.3	5.65	53.9	6.98	**
Al (mg kg^{-1})	31.2	3.04	3.1	0.12	***
Meloidogyne	29.5	5.92	55.9	4.87	***
Helicotylenchus	44.8	7.12	17.7	4.31	***
pH	6.91	0.065	5.19	0.029	***
Meloidogyne	54.8	5.86	22.7	4.33	***
Helicotylenchus	15.3	6.37	52.1	3.65	***
Soil resistivity (Ohm)	3650	143.7	1808	17.17	***
Meloidogyne	27.4	6.41	45.5	6.19	*
Helicotylenchus	58.3	7.15	33.1	6.00	**

* $P \leqslant 0.05$; ** $P \leqslant 0.01$; *** $P \leqslant 0.001$. ns = not significant.

Discussion

Our study identified *Meloidogyne* and *Helicotylenchus*, in terms of frequency of occurrence and abundance, as the dominant plant-parasitic nematode genera associated with bush tea in the northeastern part of South Africa. With regard *to Meloidogyne*, this result is not unexpected as root-knot nematodes are abundant in all crop production areas in South Africa infecting a wide variety of agri- and horticultural crops (Kleynhans *et al*., 1996; Fourie *et al*., 2015; Rashidifard *et al*., 2018). Three of the most important thermophilic *Meloidogyne* species were found either at all (*M. javanica*) or almost all (*M. incognita* and *M. enterolobii*) localities sampled during our study. In South Africa, *M. javanica* and *M. incognita* have been reported associated with many food crops, such as cereals (Mc Donald *et al*., 2017), leguminous and oil seed crops (Fourie *et al*., 2017), vegetables (Jones *et al*., 2017) and fruits (Daneel, 2017; Daneel & De Waele, 2017), as well as non-food crops including weeds (Ntidi *et al*., 2012). Both species are considered economic pests worldwide of many crops (Moens *et al*., 2009; Jones *et al*., 2013; Onkendi *et al*., 2014). Until a decade ago, *M. enterolobii* (originally reported as *M. mayaguensis*) was

only recorded in South Africa from a few crops (guava, green pepper and common blackjack) in a few provinces (KwaZulu-Natal, Limpopo and Mpumalanga; Van den Berg *et al*., 2017). Since, it is being found associated with an increasing number of crops, such as maize and potato, as well as weeds, most often in the northernmost provinces of South Africa (Mpumalanga and Limpopo), and also on peanut in Vaalharts, an irrigation scheme in the Northern Cape Province (Visagie *et al*., 2018; Collett *et al*., 2021; Marais, 2023a). The late discovery of *M. enterolobii* in South Africa (and worldwide) can be attributed to its morphological similarity with *M. incognita* and other thermophilic root-knot nematode species, which has resulted in misidentifications (Adam *et al*., 2007; Karssens *et al*., 2013). In our study, misidentification was avoided by the use of both morphological characteristics (particularly perineal pattern morphology) and the molecular SCAR-PCR technique to identify the *Meloidogyne* populations to species level. Our study confirms that *M. enterolobii* is much more widely distributed in South Africa, as was speculated by Piet Willers and Kent Kleynhans who were involved in the first report of *M. enterolobii* in South Africa (Willers, 1999; Marais *et al*., 2014). The wide distribution of *M. enterolobii* is concerning since *M. enterolobii* is considered an emerging threat worldwide to crop production (Castagnone-Sereno, 2012; Marais, 2014). It is very aggressive compared with other thermophilic *Meloidogyne* species, induces the formation of more galls, produces higher population densities, and has the ability to overcome resistance genes against various other *Meloidogyne* species (Castagnone-Sereno, 2012). Very little is known about its damage potential in South Africa, although Collett *et al*. (2021) showed that *M. enterolobii* has a faster life cycle and thus a higher reproductive potential than *M. incognita* and *M. javanica*. *Meloidogyne hapla*, a cryptophylic *Meloidogyne* species, was found at only one locality in our study. Although *M. hapla* is more common in temperate regions (Moens *et al*., 2009), in our study it was found in Levubu, together with the other three *Meloidogyne* species. It has previously been reported from warm areas in Limpopo Province on kiwi (Shokoohi & Mashela, 2020) and ferns (Marais & Swart, 2003), in Mpumalanga Province on potato (Kleynhans, 1991; Onkendi & Moleleki, 2013), and in the Douglas area of the Northern Cape Province on peach, groundnut, lucerne and potato (Marais & Swart, 2001).

Mixed populations of *Meloidogyne* species, consisting at most localities of either two or three species (but at one locality all four species were identified), occurred at all except one locality (Mbombela) where only *M. javanica* was found. Mixed *Meloidogyne* species populations are not uncommon since two or more *Meloidogyne* species are often present in the same field (Riekert & Henshaw, 1998; Karssen *et al*., 2013; Visagie *et al*., 2018).

Comparison of the morphological and molecular identification of the *Meloidogyne* populations indicated a high level of variation between the two methods. At only two out of nine localities examined did both identification methods yield the same result. At one of these localities the *Meloidogyne* population was monospecific, consisting only of *M. javanica*. At six localities, morphological identification yielded more *Meloidogyne* species compared with the molecular SCAR-PCR technique. At five of these localities, the species that was not detected by the SCAR-PCR technique was *M. incognita*, and at one locality *M. javanica*. At only one locality the SCAR-PCR technique yielded three *Meloidogyne* species (*M. javanica*, *M. incognita* and *M. enterolobii*) compared with only one *Meloidogyne* species (*M. javanica*) using morphological identification. *Meloidogyne hapla* (found at one locality) was the only species that was identified by morphological identification only, not by using the SCAR-PCR technique. All these differences illustrate that neither of the two identification methods used in our study gave a complete picture of the species composition of the *Meloidogyne* populations sampled. Morphological identification of *M. enterolobii* was not without the challenges as described by previous authors (Adam *et al*., 2007; Karssen *et al*., 2013; Visagie *et al*., 2018; Rashidifard *et al*., 2019a). In our study, the presence of *M. enterolobii* was missed at two out of nine localities examined using only identification based on the morphology of its perineal pattern. Limitations in using only the morphology of the perineal pattern for the accurate identification to species level of *Meloidogyne* populations have been emphasised previously (Karssen & Van Aelst, 2001; Carneiro *et al*., 2004; Hunt & Handoo, 2009; Onkendi *et al*., 2014). The major challenge is the similar morphology of the perineal pattern with the perineal pattern of *M. incognita* (Moens *et al*., 2009). However, examination of the morphology of the perineal pattern of the *M. enterolobii* populations found in our study show that the presence of: *i*) prominent and pronounced phasmids with fine surrounding striae; *ii*) fine striae on the lateral sides of the vulva; and *iii*) atypical perineal patterns with medium to high, pronounced square-like dorsal arches, represent valuable discriminating characteristics to distinguish *M. enterolobii* from other thermophilic *Meloidogyne* species, especially *M. incogni-*

ta (Rashidifard *et al*., 2019a). In our study, morphological identification provided a more complete picture of the species composition of the *Meloidogyne* populations under study compared with the molecular SCAR-PCR technique (in contrast with the opinion of Garcia *et al*. (2012) and Visagie *et al*. (2018) that the most decisive data for *Meloidogyne* species identification were provided by the SCAR-PCR technique). Nevertheless, a combination of identification based on both the morphology of the perineal pattern (and other morphological characteristics) and a molecular identification technique, such as SCAR-PCR, is advocated as a more accurate, complete and reliable way to identify *Meloidogyne* populations to species level (Rashidifard *et al*., 2019a, b). Accurate identification of the *Meloidogyne* species affecting bush tea is crucial for the development and application of effective management practices (Adam *et al*., 2007).

Our finding that *H. martini* is, in terms of frequency of occurrence and abundance, one of the dominant plant-parasitic nematode species associated with bush tea in the northeastern part of South Africa is unexpected. *Helicotylenchus martini* occurs in every province in South Africa but is mostly found on grasses in natural habitats (Marais, 2023a). Interestingly, *H. dihystera*, the most widely distributed *Helicotylenchus* species in South Africa (Marais, 2023a), was only found at one locality in our study. Although some species of *Helicotylenchus* have been associated with the suppression of plant growth of some crops (Subbotin *et al*., 2011), such as banana in South Africa (Daneel & De Waele, 2017), information on the damage potential of most *Helicotylenchus* species is scarce.

Pratylenchus brachyurus is another important plant-parasitic nematode that was found at most localities, albeit not consistently during both seasons, but always in low population densities. Root-lesion nematodes (*Pratylenchus* spp.) rank third only to root-knot and cyst nematodes as having the greatest economic impact on crops worldwide (Jones *et al*., 2013). In South Africa, *P. brachyurus* has been described from many hosts (Kleynhans *et al*., 1996), including banana (Daneel & De Waele, 2017), cotton (Van Biljon, 2017), maize (Mc Donald *et al*., 2017), potato (Jones *et al*., 2017) and wheat (Mc Donald *et al*., 2017). *Pratylenchus brachyurus* seems to be able to survive on bush tea but this plant species does not seem to be a good host since very low population densities of *P. brachyurus* were found in the roots.

The spiral nematodes *S. brachyurus* and *S. truncatum*, the ring nematode *D. glabrannulata* and the reniform nematode *R. parvus* were found at six or more localities in our study but in much lower population densities compared with *H. martini*. All these nematode species have a rather wide distribution in South Africa and (with the exception of *D. glabrannulata*) have been recorded from either various or a wide variety of agricultural crops and natural habitats in the country (Swart & Hugo, 1984; Fourie *et al*., 2001; Marais & Swart, 2002; Berry *et al*., 2017; Marais, 2022, 2023a). By contrast, *D. glabrannulata*, found at six localities in our study, has been recorded previously at about 15 localities in the Eastern Cape, Gauteng, Limpopo and Mpumalanga provinces in South Africa, mostly on grasses and trees in non-cultivated habitats and only once on an agricultural crop (tobacco; Marais, 2023a).

Nematode populations belonging to the *Xiphinema americanum*-group were found in the rhizosphere soil of bush tea at five localities in our study. This group of *Xiphinema* species has a wide host range in South Africa (Kleynhans *et al*., 1996; Marais & Swart, 1996, 2003) and has been listed as one of the most common and abundant plant-parasitic nematodes causing damage on grapevines and woody plants in the country (Storey *et al*., 2017). Although low population densities of the *X. americanum*-group populations were found in the rhizosphere soil of bush tea in our study, bush tea might be a good host for this dagger nematode (see further). *Xiphinema americanum* is regarded as an economically important nematode that may cause serious damage to herbal teas through direct feeding on plant roots and by transmitting plant viruses (Daramola *et al*., 2019).

When we compare the results of our study with the results of the intensive survey of nematodes associated with honeybush and rooibos in the Western Cape Province of South Africa carried out by Daramola *et al*. (2021a), several major differences can be observed. The most notable similarity is that in both studies *Meloidogyne* and *Scutellonema* are among the most frequent and abundant plant-parasitic nematode genera found in the rhizosphere soil of honeybush and bush tea. A first major difference is that *Helicotylenchus*, one of the most frequent and abundant plant-parasitic nematode genera found in the rhizosphere soil of bush tea in our study, was found infrequently and in low numbers in the rhizosphere soil of honeybush and not at all on rooibos. A second major difference is that *Xiphinema*, one of the most frequent and abundant plant-parasitic nematode genera found in the rhizosphere soil of honeybush, was recorded in only 2 and 9% of all rhizosphere soil samples of bush tea col-

lected during summer and winter, respectively. A third major difference is the absence of *P. bolivianus* in the rhizosphere soil of bush tea in our study. This highly pathogenic root-lesion nematode was recorded in 87% of all sites sampled and in high numbers on rooibos. Interestingly, it was also not found on honeybush. One can only speculate about these observed major differences. Our study was carried out in the northeastern part of South Africa while the study of Daramola *et al*. (2021a) was carried out in the southwestern part of the country differing in climate, physico-chemical soil properties, history of cultivation, *etc*. In addition, records in the SAPPNS database on honeybush samples collected in the early 2000 cite reports of *Helicotylenchus curatus*, *H. digonicus*, *Longidorus* sp., *Nanidorus renifer*, *Scutellonema bizanae*, *Xiphinema bolandium* and, *Xiphinema meridianum*, which were also not reported from bush tea (Marais, 2023b).

On honeybush, Daramola *et al*. (2021a, b) observed severe galling of the plant roots resulting in damage to the root system, which in turn resulted in severe aboveground symptoms such as nutrient deficiency, chlorosis and wilting in young plants. In some cases, severely aberrated root systems lead to plant death. Previously, Hart *et al*. (2005) had reported that farmers experienced severe constraints to honeybush cultivation due to a combination of the lack of water and the incidence of root-knot nematodes in the soil. The frequent and abundant occurrence of *Meloidogyne* on bush tea in our study identifies root-knot nematodes as the greatest nematode threat to bush tea and is of great concern for the future commercialisation of this herbal tea in South Africa.

The difference in *Meloidogyne* population densities among the nine localities is not that easy to explain. High *Meloidogyne* population densities in the rhizosphere soil seemed to occur at those localities that had, on average, the highest annual rainfall and the highest minimum temperature. This observation is not surprising since soil moisture and soil temperature are among the most important environmental factors affecting the survival and reproduction of nematodes (Song *et al*., 2017; Nisa *et al*., 2021). Since the bush tea plants were sampled in their natural habitat, they rely only on rainwater for their vegetative and reproductive growth. Bush tea plants grow more vigorously during summer when enough water is available, resulting in a more developed root system, which, in turn, produces more feeder roots and secondary roots in winter compared with summer even if rainfall is considerably less during winter. For soil-borne plant-parasitic nematodes, such as root-knot nematodes, the root tips (region of root elongation just behind the root cap and the apical meristems where the lateral roots emerge) are the preferred place to accumulate and infect their host plants (Karssen *et al*., 2013). High minimum temperatures will result in higher soil temperatures that, in turn, will favour, *inter alia*, nematode development while reproduction may continue over a longer period resulting in higher population densities. In our study, the highest in summer and among the highest *Meloidogyne* population densities in the rhizosphere soil during winter, respectively, were recorded at Levubu. At Levubu, the average annual rainfall was considerably higher compared with the other localities (100.5 *vs* > 65 mm, respectively), while the average minimum temperature (15.5°C) was the second-highest compared with most other localities. However, this positive relationship between high *Meloidogyne* population densities in the rhizosphere soil and high average annual rainfall and minimum temperature was not consistent. For example, the highest *Meloidogyne* population densities during winter were recorded at Hermansburg and Makhado (59.9 and 64.4 mm, respectively), both localities with an average annual rainfall not much different from most of the other localities; among the lowest *Meloidogyne* population densities during both summer and winter were recorded at Bushbuckridge, the locality with the highest average minimum temperature.

Soil type is another important environmental factor that may affect the occurrence and abundance of soil nematodes (Prot & Van Gundy, 1981). In our study, high *Meloidogyne* population densities in the rhizosphere soil were recorded at localities with either a light (*e.g.*, loamy-sand soil at Hermansburg) or a heavier (*e.g.*, sandy-clay soil at Levubu) soil. Although many studies reported higher *Meloidogyne* population densities in light soils with a high sand content compared with heavier soils with a higher loam and clay content (see, for instance, Prot & Van Gundy, 1981; Cadet & Thioulouse, 1998; Dabiré *et al*., 2007; Krif *et al*., 2020), a number of studies have reported the opposite. In South Africa, for example, Fourie *et al*. (2001) and Rabie (2017) reported high population densities of several *Meloidogyne* species on various crops, including soybean and pineapple, growing in soils with a high clay content.

The significant positive and negative correlations observed in our study between the RPD% of *Meloidogyne* and *Helicotylenchus* and several of the physico-chemical soil properties measured indicate that the population densities of both genera were strongly affected, albeit in dif-

ferent ways, by the levels of the nutrients Cu and K, the element Al, and the pH and soil resistivity values. Understanding the effect of edaphic factors, including physico-chemical soil properties, on the occurrence and abundance of plant-parasitic nematodes is important for the development and implementation of effective management practices (Krif *et al*., 2020). This is especially so for the management of root-knot nematodes which, together with cyst nematodes, are considered the most economically important plant-parasitic nematodes worldwide (Jones *et al*., 2013). Too high levels of plant nutrients in the soil can result in the poor uptake of essential other elements, such as iron (Fe), molybdenum (Mb) and zinc (Zn), resulting in less healthy, stressed plants that are more susceptible and sensitive to infection and damage, respectively, by soil-borne pathogens. In our study, high levels of Cu and K were positively correlated with high population densities of *Meloidogyne*. Similar positive correlations between high levels of K and high population densities of *Meloidogyne* species have been reported, for example by Asif *et al*. (2015) for *M. incognita* and *M. javanica* on tomato. Soil pH is also known to play a role in the occurrence of plant-parasitic nematodes (Noronha *et al*., 2020). Several authors have reported that a pH ranging from about 4.5 to 5.5 increases the aggregation of J2 of *Meloidogyne* at the root tips resulting in an increase in infection (Melakeberhan *et al*., 2004; Wang *et al*., 2009; Noronha *et al*., 2020). In our study, pH at the nine localities ranged from 5.4 to 6.9. Information of the effect of soil resistivity on the occurrence of nematodes in the soil is scarce. Soil resistivity is often used to study soil compaction (Seladji *et al*., 2010). Soil compaction may result in numerous changes to important soil biophysical properties such as a reduction in the availability of large pores, restriction in the movement of water and air, and a limitation of root penetration (Whalley *et al*., 1995; Seladji *et al*., 2010). All these changes may, in turn, affect important aspects of nematode-plant relationships such as nematode migration and attraction to root tips and the availability of fewer root tips for penetration and sources of food. In our study, a negative, albeit weak, correlation was observed between the population densities of *Meloidogyne* and soil resistivity.

In conclusion, our study revealed a high diversity of root-knot, spiral, root-lesion, ring, reniform as well as dagger nematodes associated with bush tea in South Africa. It represents the first and only comprehensive survey of plant-parasitic nematodes associated with bush tea. As such, all plant-parasitic nematode genera and species identified are first records for bush tea. The most frequent and abundant plant-parasitic nematodes associated with bush tea in SA (*Meloidogyne* and *Helicotylenchus*) were identified. Their damage potential on bush tea should be further examined because especially the root-knot nematodes could be a threat to the cultivation of bush tea for commercialisation. Several physico-chemical soil properties were strongly linked with the population densities of *Meloidogyne* and *Helicotylenchus*. These linkages should be further examined to evaluate the effects of these soil properties on the abundancy and thus damage potential of the dominant plant-parasitic nematodes associated with bush tea in South Africa.

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