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Occurrence and seasonal changes in the population of root-knot nematodes on honeybush (*Cyclopia* sp.)

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Summary

Root-knot nematodes in the genus *Meloidogyne* are an important group of plant-parasitic nematodes causing severe damage on agricultural crops worldwide. A study was conducted to identify the species of root-knot nematodes causing damage on honeybush monocultures and to assess the seasonal variations in the nematode population. Soil samples were collected from six experimental sites in Genadendal, Western Cape province of South Africa from 2016 to 2017. DNA was extracted from single-second stage juveniles and species identification was done using species-specific sequence-characterised amplified regions (SCAR) primers. *Meloidogyne hapla* and *M. javanica* were identified from the sites. Mean population density of the nematodes varied significantly ($p < 0.05$) in the six sites, with the peak population being recorded in summer of 2017. The study suggests that seasonal variation in temperature and moisture could contribute to changes in the population density of root-knot nematodes in the soil.

Keywords: *Meloidogyne hapla*; *Meloidogyne javanica*; population density; root-knot nematodes; seasonal fluctuation

Introduction

Honeybush (*Cyclopia* spp.) is an indigenous Cape fynbos legume plant with yellow flowers and a rich honey aroma, from which the common name is derived (Van Wyk & Gorelik, 2017). Generally, spring flowering occurs between September and October, followed by seed set in November (Motsa *et al.*, 2017). Honeybush, which is a rich source of antioxidants, possesses antimutagenic properties (Marnewick *et al.*, 2000; Kokotkiewicz & Luczkiewicz, 2009; Marnewick, 2009). The plant is popularly known for its use as a tea substitute among rural communities in the Cape Floristic Region (CFR). Traditionally, the plants, which were harvested from the wild, were processed for household use (Du Toit *et al.*, 1998; Van Wyk & Gorelik, 2017). Today, honeybush is exported internation-

ally as a health-enhancing product. To meet the growing demand for biomass, local species of honeybush are cultivated in certain areas, like the Agulhas Plain (*Cyclopia genistoides* (L.) Vent) and the Langkloof (*Cyclopia intermedia* Mey. and *Cyclopia subternata* Vogel). The sector is a source of livelihood for harvesters, small-scale farmers and commercial farmers in the Western and Eastern Cape provinces (McGregor, 2017). Commercial production of honeybush tea has risen in recent times due to increase local and international export market demands (SAHTA 2011; McGregor, 2017). However, reduction in yield and damage caused by plant-parasitic nematodes is one of the main problems confronting the local tea industry.

Root-knot nematodes (RKN) are important pests of agricultural crops worldwide. They are widespread on leguminous and flower-

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ing plants, consisting of about 98 species (Jones *et al.*, 2013), of which about 22 species have been described from Africa (Onkendi *et al.*, 2014). *Meloidogyne arenaria* Chitwood, *Meloidogyne incognita* (Kofoed & White) Chitwood and *Meloidogyne javanica* (Treb) Chitwood are usually found in the warm, tropical and subtropical regions of the world, while *Meloidogyne chitwoodi* Golden, O'Bannon, Santo & Finley, *Meloidogyne fallax* Karssen and *Meloidogyne hapla* Whitehead are more common in the temperate regions (Moens *et al.*, 2009). In South Africa, *M. incognita* and *M. javanica* have been declared as economic pests of most crops, with about 4000 host plants, including field crops, ornamentals, medicinal and aromatic plants as well as weeds (Fourie *et al.*, 1998; 2012; ARC 2014). The four major species, which include *M. incognita*, *M. javanica*, *M. arenaria* and *M. hapla* and the newly emerging *Meloidogyne enterolobii* Yang & Eisenback, 1983 have all been reported throughout South Africa, irrespective of the climatically different regions of the country concerned. *Meloidogyne javanica* has been reported from grapevine in the Western Cape province (Coetzee, 1968; Loubser, 1988), with there being indications that *M. javanica* is the most common species, followed by *M. incognita*, *M. arenaria* and *M. hapla*.

Seasonal fluctuations in the population of RKN on important agricultural crops worldwide have been documented (Chormule *et al.*, 2017; El-Ghonaimy *et al.*, 2015; Fayzia *et al.*, 2018; Shokoohi *et al.*, 2019). Variation in soil temperature and moisture, due to seasonal changes are key factors that contribute mainly to the spatial and temporal distribution of nematodes in the soil (McSorley, 1998;

Dinardo-Miranda & Fracasso, 2010). An understanding of the seasonal fluctuations of RKN on honeybush plants will, therefore, help in designing appropriate intervention plans for nematode control. Accurate identification of nematode pests is also key to the effective management and the quarantine strategies employed to prevent their spread. The traditional morphological identification, which is based on the morphological features, the morphometrics of second-stage juveniles (J2) and the perineal patterns of the adult female, is usually difficult, with it often showing wide intraspecific variation, with unreliable values (Subbotin *et al.*, 2015). The use of species-specific primers, however, offers a relatively easy and rapid diagnostic tool for identifying the *Meloidogyne* species, and can be done using J2, which tends to be readily available in the soil. Therefore, the aim of this present investigation was to study the association of RKN with honeybush tea plants and to assess the seasonal changes in the nematode population on honeybush monocultures in the Western Cape province of South Africa.

Materials and Methods

Site description

Genadendal is located about 120 km east of Cape Town at -34.051565, 19.517402, and at an altitude of 231 m above sea level. The sites of honeybush cultivation included in the study were: a planting trial located about 5 km west of Genadendal Site A, which is composed of three plots (A1, A2 and A3), and other three plots (Sites B, C and D), which are situated in the main town in the area.

Fig. 1. Google Earth image showing the location of the sampling sites in the Western Cape province of South Africa.

Site A was an abandoned honeybush field, where three species of honeybush had been planted in 2007 (Fig. 1). The species involved were *C. maculata*, *C. genistoides*, and *C. subternata*. Three other honeybush plots, planted as part of a different experimental trial in 2015, with the same three species of honeybush, were located in Genadendal. The sites concerned (B, C and D) were also sampled for RKN. Mild winter rainfall between May and August is typical of the Mediterranean-type climate in the region, as are hot, dry summers, in which temperatures reach up to 42°C. The annual rainfall in the region reaches about 520 mm per annum. The plants involved were cultivated under dry land conditions. The climatic conditions for the weather stations that was closest to the geographical area was obtained from the Agro meteorology (ARC-ISCW) weather station at Elsenburg, Stellenbosch.

Sampling, nematode isolation and identification

Sampling was done on three plots on the abandoned honeybush farmland and other three experimental plots in Genadendal between September 2016 and July 2017. Soil samples were collected from the rhizosphere of the honeybush plants, up to a depth of about 50 cm. Five plants were sampled per plot, with the soil samples from each plot being bulked and mixed, after which a composite sample was taken for analysis. Galled root samples were also collected from infected plants, while dead plants were uprooted and transported to the laboratory to assess the extent of root damage. Nematodes were extracted from the soil samples, using a modified Baermann extraction funnel method (Cobb, 1918). This involved pouring the samples over a two-ply paper towel that was supported on a coarse-meshed plastic screen and placed over a metal dish. The process was repeated for all the samples. Second-stage juveniles (J2) were collected from the extraction tray after 24 h and

were examined under a stereo microscope at 10X. Root samples were washed and cut up into small pieces, with 50 g of the root samples being macerated in a blender for 60 s and poured onto a piece of filter paper placed on an extraction tray (Whitehead and Hemming 1965). The nematode suspension containing the J2 of RKN were collected from the tray into 250ml beakers after 24 h, and concentrated into 20ml from which an aliquot of 1ml was taken for nematode count. Mean population density (MPD) of nematodes per 250ml of each sample were recorded throughout the sampling period. Analysis of variance (ANOVA) was performed on the MPD of the RKN and the total number of plant-parasitic nematodes using SPSS version 26 (Statistical Package for the Social Sciences). Identification and counting of nematodes was done using a Leica DM2000 compound microscope at 40X.

DNA extraction and polymerase chain reaction

DNA was extracted from single J2 root-knot larvae obtained from the honeybush samples as described by Daramola *et al.* (2020). The specimen for DNA extraction were placed in a Petri-dish and washed twice with ddH₂O. Single J2 specimens were each cut into 2 – 3 parts and placed in 10 µl lysis buffer (500 mM MgCl₂, 10 mM DTT, 4.5 % Tween20®, 0.1 % gelatine and 3 µl proteinase K at 600 µg ml⁻¹), which was placed on the side of an Eppendorf tube. The tubes were kept at -80°C for about 15 min, and then incubated in a thermocycler at 65°C for 1 h, and at 95°C for 15 min, to lyse the cells and digest the proteins completely. The extraction yielded DNA products from individual J2, which were further used for polymerase chain reaction.

PCR for the amplification of the DNA samples was done using KAPA2G™ 40 Robust HotStart ReadyMix (KAPA Biosystems), with specific primers in a 25 µL reaction consisting of 5 µL DNA

Table 1. Temperature and rainfall distribution around Genadendal, Western Cape province (2016 – 2017).

Month	2016			2017		
	Temperature °C (max)	Temperature °C (min)	Rainfall (mm)	Temperature °C (max)	Temperature °C (min)	Rainfall (mm)
January	32.37	19.34	0.26	29.89	16.29	1.4
February	30.59	16.82	0.34	30.73	17.48	0.14
March	26.95	16.06	1.11	30.09	15.07	0.09
April	26.08	13.63	0.89	28.79	14.34	0.92
May	24.31	11.22	0.34	25.11	11.79	0.33
June	20.03	8.48	2.05	20.44	7.51	0.82
July	18.68	8.2	2.72	18.96	7.09	0.4
August	21.55	8.83	1.47	17.28	7.55	1.65
September	21.06	9.34	0.77	20.56	8.66	0.58
October	25.74	11.23	0.25	22.1	9.37	0.79
November	28.04	13.99	0.18	24.27	12.5	1.27
December	31.05	16.07	0.19	26.92	14.98	0.27

Source: Agricultural Research Council, Stellenbosch, Western Cape South Africa.

Fig. 2. Mean population density of root-knot nematode (RKN) and other plant-parasitic nematode (PPN) associated with honeybush cultivation on six sites at Genadendal, Western Cape province of South Africa (2016-2017). Error bars represent standard errors ($\pm$ SE).

and 2.5 μ L each of the primer combination. A positive sample of *M. javanica* DNA sample from potato was included as control. The PCR reactions were centrifuged at 15,071 RCF at 10°C for 2 min, which were then placed in the thermocycler (GeneAmp 2720). The cycling condition for each primer set is as described by Adam *et al.* (2007). PCR products were separated on 1.5 % agarose gel in a Tris borate (TBE) buffer and visualised under UV light, using a trans-illuminator imaging system, after staining with ethidium bromide.

Ethical Approval and/or Informed Consent

This article does not contain any studies with human participants or animals by any of the authors.

Results

Temperature and rainfall pattern at the sampling sites are indicated in Table 1. The location of the honeybush farms and the experimental plots that were sampled in the current study is shown in Fig. 1. Slight changes in the average temperature were observed during the sampling period. The summer of 2016 was recorded as having the highest temperature, with an average of 31.05°C and a low precipitation of 0.19 mm. Low rainfall was recorded during the sampling period, due to the drought that was experienced in the Western Cape at the time. Maximum precipitation of 1.65 mm was recorded during the winter of 2017. The annual amount of rainfall recorded for 2017, which was about 251.7 mm, was very low when compared with that which was recorded for the preceding years (Table 1).

Table 2. Primer codes used for identification of *Meloidogyne* species, sequences and sources.

Species	Primer	Sequence 5'-3'	Source
<i>M. arenaria</i>	Far	TCGGCGATAGAGGTAAATGAC	<i>M. arenaria</i> -specific SCAR Zijlstra <i>et al.</i> (2000)
	Rar	TCGGCGATAGACACTACAAACT	
<i>M. javanica</i>	Fjav	GGTGCGCGATTGAACTGAGC	<i>M. javanica</i> -specific SCAR Zijlstra <i>et al.</i> (2000)
	Rjav	CAGGCCCTTCAGTGGAATATAC	
<i>M. incognita</i>	MI-F	GTGAGGATTCAGCTCCCCAG	<i>M. incognita</i> -specific SCAR Meng <i>et al.</i> (2004)
	MI-R	ACGAGGAACATACTCTCCGTCC	
<i>M. hapla</i>	JMV1	GGATGGCGTGCTTTCAAC	<i>M. hapla</i> -, <i>M. chitwoodi</i> -and <i>M. fallax</i> -specific IGS-SCAR Wishart <i>et al.</i> (2002)
	JMV2	TTTCCCCTTATGATGTTTACCC	
	JMVhapla	AAAAATCCCCTCGAAAAATCCACC	

Adam *et al.*, 2007

Fig. 3. Mean population density of root-knot nematode (RKN) associated with honeybush cultivation at six sampling sites in Genadendal, Western Cape province of South Africa (2016-2017). Error bars represent standard errors ($\pm$ SE).

Seasonal changes in population density of RKN associated with honeybush

The population density of plant-parasitic nematodes found in association with the honeybush during the sampling period is shown in Figure 2. The peak nematode population for all plant-parasitic nematodes was recorded in the summer of 2017 with a mean population density of 7,780 nematodes per 250ml of soil. There was a consistent increase in the population of the RKN from winter of 2016, until the end of sampling period in July 2017. The peak nematode population for RKN was recorded in the winter of 2017 at 5,280 RKN per 250ml soil.

The population density of the RKN found in association with the honeybush between 2016 and 2017 is shown in Figure 3. The effects of the seasonal variation on the mean nematode density (MPD) of RKN nematodes from the six sampling sites are shown in Figure 4. An increase was found in the MPD of the RKN from September 2016 to July 2017. However, no significant difference ($p < 0.05$) was found in the effect of the sampling period on the population of the RKN recorded as being present on the honeybush plots. The mean population density of the RKN present on the varied honeybush plots was compared. A significant difference ($p < 0.05$) was found in the number of RKN recorded at the different sampling

sites. The mean nematode population, which varied significantly ($p < 0.005$) in the infected honeybush fields, followed a consistent pattern throughout the sampling period (Fig. 3). Significantly ($p < 0.05$) higher numbers of nematodes were recorded in the soil samples taken from site D throughout the sampling period. Low numbers of RKN were recorded from sampling site C, although the numbers were not significantly different ($p = 0.828$) from those that were recorded as being present at sites A1 and A3, which were lower than those that were obtained from sampling sites A2 and B. The effect of interaction between the sampling period and the honeybush sites regarding the RKN population are compared in Figure 3. The sampling period had no significant effect ($p > 0.05$) on the population of RKN recorded from the sampling sites. Although peak nematode population was recorded from site D during the summer (February) of 2017 however, this was not significantly different from the population that was recorded in the following winter. Also, no RKN was recorded as being present on sampling site C at the onset of the sampling period in September 2016, however, low numbers of the nematode were recorded at the conclusion of the period concerned, which indicates that the nematodes must have spread into the field.

Fig. 4. Seasonal fluctuation and changes in the nematode population during the sampling period of 2016-2017 at the six experimental sites in Genadendal, Western Cape province of South Africa.

Symptoms and damage caused by root-knot nematodes on honeybush

The damaging effects of RKN on honeybush plants are shown in Figure 5, with the symptoms of such damage varying with the age of the plants. In the case of the older plants, heavily galled roots were observed, and the plants showed above-ground symptoms of nutrient deficiency and chlorosis. Wilting was more common among the younger infected plants, with the plants losing their foliage, and the root system becoming completely damaged and

non-functional. In some cases, the loss of plant stands was recorded. Figure 6 shows the persistence of healthy flowering honeybush plants and the loss of plant stands found on the abandoned honeybush farm.

Molecular identification

Meloidogyne hapla and *M. javanica* were identified from the honeybush plots, with DNA amplification using species-specific SCAR primers (Table 2). The results of the SCAR-based molecular iden-

Fig. 5. Symptoms of RKN damage on honeybush roots.

Fig. 6. A-B: Healthy flowering honeybush plants. C-D: Nematode damage on honeybush field, showing loss of plant.

tification of the J2 of RKN isolated from the six honeybush plots indicated that *M. javanica* and *M. hapla* were evident in the sampled fields. The presence of *M. javanica* was recorded on only one of the experimental plots (Site A3) out of the six samples that were amplified, whereas *M. hapla* was more prevalent on the abandoned honeybush farm. The SCAR primers, Fjav/Rjav (*M. javanica*) and JMV1/JMV2/ JMV (*M. hapla*), provided consistent results, with the PCR products being readily amplified from using a single J2 (Fig. 7), therefore providing a rapid and simple approach for detecting *Meloidogyne* species.

Discussion

The results of the current study showed that there is a strong association between the RKN species (*M. hapla* and *M. javanica*) and honeybush plants with the nematodes possessing the ability to cause serious damage and economic losses to the plants concerned. Economic losses and severe damage to many field and horticultural crops, due to RKN, have received global attention (Moens *et al.*, 2009; He *et al.*, 2015). *Meloidogyne* species have been described as one of the top ten plant-parasitic nematodes worldwide (Jones *et al.*, 2013), associated with a staggering

estimated annual losses of \$157 billion (USD), globally (Abad *et al.*, 2008). In Africa, RKN have been described as posing a major threat to food security, with serious implications for the gross national income and for the foreign exchange earnings involved. Coyne *et al.* (2018) describe RKN as probably posing the greatest biotic threat to agriculture in sub-Saharan Africa. The results of the current study confirm the damaging potential of the nematodes for the monoculture of honeybush, thus indicating a potential threat to the tea industry. In the current investigation, the extensive root damage caused by RKN could have contributed to the complete crop failure that was recorded on the abandoned honeybush tea plantation.

The above-ground symptoms of the infection of the young honeybush plants observed in the current study are synonymous with the symptoms that have frequently been associated with *Meloidogyne* infection elsewhere (Mitkowski & Abawi, 2003). Such symptoms include stunting, the yellowing of leaves and wilting, which can, eventually, result in a reduction in the quality and quantity of yield, especially in terms of honeybush production, where the aerial parts and foliage of the plants are harvested for tea production. The fact that high numbers of the nematodes were found in the soil (2340/250 ml⁻¹ soil) indicates the serious threat and

Fig. 7: Gel pictures obtained from the amplification of DNA products of single J2s of RKN from honeybush plots. a. *Meloidogyne javanica* (amplified with FJav/Rjav primers) and b. *M. hapla* (with JMV1/JMV2 & JMV primers).

damage caused to the plants concerned, with the economic losses associated with *Meloidogyne* species having the potential to be extensive in areas suffering from high infestation (Onkendi & Moleleki, 2013).

The four important species of RKN, namely *M. incognita*, *M. arenaria*, *M. javanica* and *M. hapla*, have been reported in association with a wide range of agricultural crops and weeds in South Africa (Kleynhans *et al.*, 1996; Ntidi *et al.*, 2015; Marais, 2015; Agenbag, 2016). The distribution of the nematode species has been linked to their adaptability to the prevailing climatic conditions and to the availability of suitable host plants. From earlier reports in the Western Cape province of South Africa, *M. javanica* can be seen to be more frequently encountered, and pathogenic, in the region. Daramola *et al.* (2020) reported *M. javanica* and *M. hapla* from honeybush fields in Western Cape. According to Loubser and Meyer (1987), the presence of the two most important species in

South Africa vineyards is determined by the vastly different climatic regions concerned. The results of the study showed that *M. hapla* was more prevalent in the sampled honeybush plantations in the Western Cape. Whereas *M. javanica* was only found to be present in one field, the presence of *M. incognita* and *M. arenaria* was not recorded on the honeybush plots. An earlier report indicates that the farmers were experiencing severe constraints to honeybush cultivation, due to the lack of water and to the incidence of RKN in the soil (Hart *et al.*, 2005). As a result, the drought experienced during the sampling period could have predisposed the plants to more extensive damage.

Edaphic factors as soil type, temperature and rainfall are key factors that help to determine the distribution of the *Meloidogyne* species. Soil temperature and moisture are considered the most important abiotic factors affecting nematode population dynamics (McSorley, 1998). In the current investigation, the population den-

sity of the nematodes varied significantly in the different sites and high population of RKN were observed on infected sites throughout the sampling period, even at low temperatures during winter. *Meloidogyne hapla* seems to favour the temperate and colder regions, with it being able to survive in field temperatures below 0°C (Desaeger, 2019). Unlike the thermophilic species, of warm climatic areas, they can withstand colder temperatures and can, occasionally, be found in the cooler upland tropics (Brodie *et al.*, 1993). In the current study, the presence of *M. hapla* was recorded in Genadendal, which is a town that is located in a relatively temperate region of South Africa, where temperatures can sometimes drop to below 5°C during the winter months. The fact that the peak population of the nematode was recorded during the winter of the sampling period suggests that *M. hapla* can survive at low temperatures, and that it is well adapted to the climatic conditions concerned. The annual rainfall of 273mm in the region, and the sandy clay soil type that is common in the Western Cape, seem to also provide ideal conditions in which the nematodes can thrive. At the outset of the sampling period in the current study, four honeybush plots were initially found to be infested, but the presence of RKN nematodes was recorded on all six plots at the end of the sampling period concerned. The above also indicates that RKN can easily spread to other fields, with it further emphasising the need to ensure compliance with good agronomic practices and to implement plant quarantine measures on the infested fields, so as to prevent the devastating effect of root-knot damage being caused to the plants.

The DNA amplification of the J2s using SCAR primers identified *M. hapla* and *M. javanica* as being species associated with the inflicting of damage on honeybush plants. A similar result was earlier obtained by Adam *et al.* (2007), who proposed the molecular diagnostic key for the identification of seven *Meloidogyne* species using J2. The above agrees with the findings of Fourie *et al.* (2001), which identified and differentiated, by means of the SCAR-PCR technique, that *M. fallax*, *M. chitwoodi*, *M. javanica*, *M. incognita*, *M. arenaria* and *M. hapla* were species of RKN occurring in South Africa. The accurate identification of RKN species is important for the design of effective nematode management options, and for implementing phytosanitary regulations. According to Powers and Harris (1993), identification methods using the J2 are useful for making effective crop management decisions. The methods are also rapid and cost-effective to use, thereby making diagnosis affordable to smallholder farmers. Therefore, the molecular identification of the *Meloidogyne* species, based on amplification of the J2 with species-specific primers, is encouraged and SCAR primers should be developed to accommodate species delimitation of other important plant-parasitic nematodes of agricultural crops.

Conflict of Interest

Authors have no potential conflict of interest pertaining to this submission to Helminthologia.

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References

- ABAD, P., GOUZY, J., AURY, J.M., CASTAGNONE-SERENO, P., DANCHIN, E.G.J., DELEURY, E., PERFUS-BARBECH, L., ANTHOUARD, V., ARTIGUENAVE, F., BLOK, V.C., *et al.* (2008): Genome sequence of the metazoan plant-parasitic nematode *Meloidogyne incognita*. *Nat Biotechnol*, 26: 909 – 915. DOI: 10.1038/nbt.1482
- ADAM, M.A.M., PHILLIPS, M.S., BLOK, V.C. (2007): Molecular diagnostic key for identification of single juveniles of seven common economically important species of root-knot nematode (*Meloidogyne* spp.). *Plant Pathol*, 56: 190 – 197. DOI: 10.1111/j.1365-3059.2006.01455.x
- AGENBAG, M. (2016): *Identification and reproduction potential of South African Meloidogyne species*. MSc dissertation, South Africa, Potchefstroom, North-West University.
- AGRICULTURAL RESEARCH COUNCIL (2014): *Nematodes*. Retrieved July 22, 2020 from: [https://www.arc.agric.za/arc-vopi/Pages/Crop %20Protection/Nematodes.aspx](https://www.arc.agric.za/arc-vopi/Pages/Crop%20Protection/Nematodes.aspx)
- BRODIE, B.B., EVANS, K., FRANCO, J. (1993): Nematode Parasites of Potatoes In: EVANS, K., TRUDGILL, D.L., WEBSTER, J.M. (Eds) *Plant parasitic nematodes in temperate agriculture*. CABI Publishing. Wallingford, UK, pp. 87 – 132
- COBB, N.A. (1918): *Estimating the nematode population of soil*. Agricultural Technical Circular, Bureau of Plant Industries, United States Department of Agriculture, no. 1, 48pp.
- CHORMULE, A.J., MHASE, N.L., KULKARNI, S.R., GURU, P.N. (2017): Seasonal incidence of *Meloidogyne incognita* infesting Grape in field. *Ann Plant Prot Sci*, 25(1): 198 – 201
- COETZEE, V. (1968): The distribution of the family Heteroderidae (Filipjev, 1934) in South Africa and some host records of *Meloidogyne* species. *S Afr J Agric Sci*, 11: 775 – 788
- COYNE, D.L., CORTADA, L., DALZELL, J.J., CLAUDIUS-COLE, A.O., HAUKELAND, S., LUAMBANO, N., TALWANA, H. (2018): Plant parasitic nematodes and food security in sub-Saharan Africa. *Annu Rev Phytopathol*, 56: 381 – 403. DOI: 10.1146/annurev-phyto-080417-045833
- DARAMOLA, F.Y., MALGAS, R., MALAN, A.P. (2020): First record and molecular identification of root-knot nematodes from honeybush tea plants (*Cyclopia* spp.) in South Africa. *Nematology*, 22(2020): 591 – 593. DOI: 10.1163/15685411-bja10023
- DESAEGER, J. (2019): *Meloidogyne hapla*, the northern root-knot nematode in Florida strawberries and associated double-cropped vegetables (ENY-070/IN1224, 10/2018) *UF/IFAS Extension*, University of Florida, Gainesville, FL. Retrieved July 22, 2020 from

<https://journals.flvc.org/edis/article/view/106583>

- DINARDO-MIRANDA, L.L., FRACASSO, J.V. (2010): Spatial and temporal variability of plant-parasitic nematodes population in sugarcane. *Bragantia*, 69: 39 – 52. DOI: 10.1590/S0006-87052010000500006
- DU TOIT, J., BRITZ, T.J., JOUBERT, E. (1998): Honeybush tea—a rediscovered indigenous South African herbal tea. *J Sustain Agr*, 12(2-3): 67 – 84. DOI: 10.1300/J064v12n02_06
- EAPEN, S.J. (1993). Seasonal variations of root-knot nematode population in a cardamom plantation. *Indian J Nematol*, 23(1): 63 – 68
- EL-GHONAIMY, A.M., EL-NAGDI, W.M.A., YOUSSEF, M.M.A. (2015): Seasonal variations of population density of root-knot nematode, *Meloidogyne incognita* as affected by different cropping sequences. *Sci Agric*, 10(1): 35 – 37
- FAYZIA, M.M., AMIRA, S.S., KHALIL, A.E.M., SHAWKKY, S.M., TAHER, A.A. (2018): Seasonal fluctuation and biological control of root-knot nematodes *Meloidogyne incognita* on cucumber. *Pak J Nematol*, 36(1): 71 – 81. DOI: 10.18681/pjn.v36.i01.p71-81
- FOURIE, H., ZIJLSTRA, C., MCDONALD, A.H. (1998): ITS-PCR sequence based identification of *M. chitwoodi* and screening of crops for host suitability. *Afr Plant Prot*, 4: 107 – 111
- FOURIE, H., ZIJLSTRA, C., MCDONALD, A.H. (2001): Identification of root-knot nematode species occurring in South Africa using the SCAR-PCR technique. *Nematology*, 3: 675 – 680. DOI: 10.1163/156854101753536046
- GNANAPRAGASAM, N.C., MOHOTI, K.M. (2005): Nematode parasites of tea. In Luc, M., Sikora, A., Bridge, J. (Eds) *Plant parasitic nematodes in subtropical and tropical agriculture*. CABI Publishing, Wallingford, UK pp. 581 – 609
- HART, T., BURGESS, R., BEUKES, O., HART, C. (2005): Reducing pitfalls in agricultural development projects: a case for the participatory project management cycle (ppmc). *S. Afr. j. agric. ext.*, 34(1): 104 – 121
- YE, W., ZENG, Y., KERNS, J. (2015). Molecular characterisation and diagnosis of Root-knot nematodes (*Meloidogyne* spp.) from Turf-grasses in North Carolina, USA. *PLoS One*. 24; 10 (11): e0143556. doi: 10.1371/journal.pone.0143556
- JONES, J.T., HAEGEMAN, A., DANCHIN, E.G.J., GAUR, H.S., HELDER, J., JONES, M.G.K., KIKUCHI, T., MANZANILLA-LÓPEZ, R., PALOMARES-RISUS, J.E., WESEMAEL, W.M.L., et al. (2013): Top 10 plant-parasitic nematodes in molecular plant pathology. *Mol Plant Pathol*, 14: 946 – 961. DOI: 10.1111/mpp.12057
- KAMUNYA, S.M., WACHIRA, F.N., LANG'AT, J., OTIENO, W., SUDO, V. (2008): Integrated management of root knot nematode (*Meloidogyne* spp.) in tea (*Camellia sinensis*) in Kenya. *Int J Pest Manag*, 54(2): 129 – 136. DOI: 10.1080/09670870701757896
- KARSSSEN, G. (2002): *The plant-parasitic nematode genus Meloidogyne Göldi, 1892 (Tylenchida) in Europe*. Leiden, Netherlands, Brill.
- KLEYNHANS, K.P.N., VAN DEN BERG, E., SWART, A., MARAIS, M., BUCKLEY, N.H. (1996): *Plant nematodes in South Africa. Plant Protection Research Institute Handbook No. 8*. Agricultural Research Council – Plant Protection Research Institute, Pretoria.
- KOKOTKIEWICZ, A., LUCZKIEWICZ, M. (2009): Honeybush (*Cyclopia* sp) – a rich source of compounds with high antimutagenic properties. *Fitoterapia*, 80: 3 – 11. DOI:10.1016/j.fitote.2008.11.001
- LOUBSER, J.T. (1988): Occurrence and pathogenicity of root-knot nematodes (*Meloidogyne* species) in South African vineyards. *S. Afr. J. Enol. Vitic.*, 9: 21 – 27
- LOUBSER, J.T., MEYER, A. J. (1987): Population dynamics of the Root-knot Nematodes *Meloidogyne incognita* (Kofoid & White) Chitwood and *M. javanica* (Treub) Chitwood on Grapevines in two different Regions of South Africa. *S. Afr. J. Enol. Vitic.*, 8 (2): 36 – 40. DOI: 10.21548/8-2-2312
- MARAI, M. (2015): *Identification job sheet N03655: dataset from South African Plant-Parasitic Nematode Survey (SAPPNS) database*. Pretoria, South Africa, Nematology Unit, Biosystematics Division, Plant Protection Research Institute, Agricultural Research Council.
- MARNEWICK, J.L. (2009): Rooibos and honeybush: recent advances in chemistry, biological activity and pharmacognosy. *African Natural Plant Products: New Discoveries and Challenges in Chemistry and Quality. ACS Symposium series*, 1021: 277 – 294. DOI:10.1021/bk-2009-1021.ch016
- MARNEWICK, J., GELDERBLUM, W., JOUBERT, E. (2000): An investigation on the antimutagenic properties of South African herbal teas. *Mutat Res Genet Toxicol Environ Mutagen*, 471(1): 157 – 166. DOI: 10.1016/S1383-5718(00)00128-5
- MCGREGOR, G. (2017): *An overview of the honeybush industry* (EADP 696) Department of Environmental Affairs and Development Planning, Cape Town. Retrieved July 22, 2020 from https://www.westerncape.gov.za/eadp/files/atoms/files/eadp696_an_overview_of_the_honeybush_industry_may2017_0.pdf
- MCSORLEY, R. (1998): Population Dynamics. In BARKER, K.R., PEDERSON, G.A., WINDHAM, G.L., BARTELS, J., HATFIELD, J.M., BAENZIGER, P., BIGHAM J. (Eds) *Plant and Nematode Interactions*. American Society of Agronomy, Madison, Wisconsin, USA, pp. 109 – 133
- MENG, Q.P., LONG, H., XU, J.H. (2004): PCR assays for rapid and sensitive identification of three major root-knot nematodes, *Meloidogyne incognita*, *M. javanica* and *M. arenaria*. *Zhi Wu Bing Li Xue Bao*, 34: 204 – 210
- MITKOWSKI, N.A., ABAWI, G.S. (2003): Root-knot nematodes. *Plant Health Instr*, DOI: 10.1094/PHI-I-2003-0917-01
- MOENS, M., PERRY, R.N., STARR, J.L. (2009): *Meloidogyne* species – a diverse group of novel and important plant parasites. In PERRY, R.N., MOENS, M., STARR, J.L. (Eds) *Root-knot nematodes*. CABI Publishing, Wallington, UK, pp. 1 – 7
- MOTSA, M.M., SLABBERT, M.M., BESTER, C., NGWENYA, M.Z. (2017): Phenology of honeybush (*Cyclopia genistoides* and *C. subternata*) genotypes. *S Afr J Bot*, 110: 57 – 67. DOI:10.1016/J.SAJB.2016.03.005
- NTIDI, K.N., FOURIE, H., MCDONALD, A.H., DE WAELE, D., MIENIE, C.M.S. (2012): Plant parasitic nematodes associated with weeds in developing agriculture in South Africa. *Nematology*, 1: 875 – 887

- ONKENDI, E.M., MOLELEKI, L.N. (2013): Detection of *Meloidogyne enterolobii* in potatoes in South Africa and phylogenetic analysis based on intergenic region and the mitochondrial DNA sequences. *Eur J Plant Pathol*, 136: 1 – 5. DOI: 10.1007/s10658-012-0142-y
- ONKENDI, E.M., KARIUKI, G.M., MARAIS, M., MOLELEKI, L.N. (2014): The threat of root-knot nematodes (*Meloidogyne* spp.) in Africa: a review. *Plant Pathol*, 63: 727 – 737. DOI: 10.1111/ppa.12202
- ORISAJO, S.B. (2013): Effect of plant-parasitic nematodes associated with tea in Nigeria. *Afr J Basic Appl Sci*, 5: 184 – 187. DOI: 10.5829/idosi.ajbas.2013.5.4.1634
- POWERS, T.O., HARRIS, T.S. (1993): A polymerase chain reaction method for identification of five major *Meloidogyne* spp. *J Nematol*, 25: 1 – 6
- SHOKOOHI, E., MASHELA, P.M., IRANPOUR, F. (2019): Diversity and seasonal fluctuation of tylenchid plant-parasitic nematodes in association with alfalfa in the Kerman Province (Iran). *J Nematol*, 51: 1 – 14. DOI: 10.21307/jofnem-2019-074
- SOUTH AFRICAN HONEYBUSH TEA ASSOCIATION (2011): *A profile of the South African honeybush tea market value chain*. South Africa, Department of Agriculture, Forestry and Fisheries, pp. 6 – 9 Retrieved March 23, 2020 from <https://www.nda.agric.za/doiDev/sideMenu/Marketing/Annual%20Publications/Commodity%20Profiles/INDUSTRIAL%20PRODUCTS/Honeybush%20Tea%20Market%20Value%20Chain%20Profile%202011-12.pdf>
- SUBBOTIN, S.A., VOVLAS, N., YEATES, G.W., HALLMANN, J., KIEWNICK, S., CHIZHOV, V.N., MANZANILLA-LOPEZ, R.H., INSERRA, R.N. & CASTILLO, P. (2015): Morphological and molecular characterisation of *Helicotylenchus pseudorobustus* (Steiner, 1914) Golden, 1956 and related species (Tylenchida: Hoplolaimidae) with a phylogeny of the genus. *Nematology*, 17: 27 – 52
- VAN, WYK, B.-E., GORELIK, B. (2017): The history and ethnobotany of Cape herbal teas. *S Afr J Bot*, 110: 18 – 38. DOI: 10.1016/j.sajb.2016.11.011
- WISHART, J., PHILLIPS, M.S., BLOK, V.C. (2002): Ribosomal intergenic spacer: a polymerase chain reaction diagnostic for *Meloidogyne chitwoodi*, *M. fallax*, and *M. hapla*. *Phytopathology*, 92: 884 – 92. DOI: 10.1094/PHYTO.2002.92.8.884.
- WHITEHEAD, A.G., HEMMING, J.R. (1965): A Comparison of some quantitative methods extracting small vermiform nematodes from the soil. *Ann Appl Biol*, 55: 25 – 38. DOI: 10.1111/j.1744-7348.1965.tb07864.x
- YANG, B., EISENBACK, J.D. (1983): *Meloidogyne enterolobii* n. sp. (Meloidogynidae), a root-knot nematode parasitizing pacara ear-pod tree in China. *J Nematol*, 5: 381 – 391
- ZIJLSTRA, C. (2000): Identification of *Meloidogyne chitwoodi*, *M. fallax* and *M. hapla* based on SCAR-PCR a powerful way of enabling reliable identification of populations or individuals that share common traits. *Eur J Plant Pathol*, 106: 283 – 90. DOI: 10.1023/A:1008765303364