

Nematode assemblages in grain crop fields in South Africa

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ABSTRACT

Grain crop yields and quality are adversely affected by infection and parasitism of plant-parasitic nematodes; especially root-knot (*Meloidogyne*) and lesion nematodes (*Pratylenchus*) that dominate in local production areas. Local grain producers experience poor crop performance and yield decline despite optimal management practices being implemented, e.g., sufficient fertilizer application, proper disease and pest control, and others. Hence they requested this study of which the aim was to determine the abundance and diversity of plant-parasitic and beneficial nematodes in 18 fields identified. Root and rhizosphere samples of maize, soybean, sunflower, and wheat were obtained from the 18 fields during two subsequent summer growing seasons 2020 for Survey 1 and 2021 for Survey 2) were grown on fields investigated. Motile life stages and eggs and second-stage juveniles (J2) of the sessile endoparasitic genera *Meloidogyne* and *Rotylenchulus* were extracted from 5 g and 50 g roots, respectively, using standardized procedures. Similarly, specialised and accredited techniques were used to extract motile individuals of both plant-parasitic and beneficial nematodes from soil samples. Prominence values (PV) were calculated for all nematode genera and/or families, while univariate analyses (Repeated Measures ANOVA) were done to elucidate the population dynamics of predominant plant-parasitic nematode genera/families. Multivariate data analyses (Principal Component Analyses) examined whether associations existed between nematodes and selected abiotic factors. In 50 g roots *Meloidogyne* dominated, followed by *Rotylenchulus* (PV of 8915 and 978 eggs and J2, respectively). Morphological and molecular species identification for *Meloidogyne* evidenced *M. incognita* as the predominant species, followed by *M. javanica* and *M. arenaria*; *M. enterolobii* were not identified. These results are crucial for proper planning of grain crop production in fields where mostly mixed communities of the genus occur. *Meloidogyne* and *Rotylenchulus* also dominated in 5 g roots, followed by migratory lesion nematodes. In soil, *Pratylenchus* was predominant followed by the ectoparasitic families Dolichodoridae and Criconeematidae. This shift in predominant plant-parasitic nematode genera occurring in soils of grain fields investigated in this study is interesting and novel; it accentuates producers to implement proactive measures to mitigate damage that can be caused by the predominant ectoparasites. Densities of predominant plant-parasitic nematodes in grain crop rhizospheres differed significantly for the two seasons, with multivariate analyses not portraying that any of the selected abiotic parameters included may have contributed to this phenomenon.

Exceptionally low levels of beneficial nematodes were present in soils from 17 of the 18 fields sampled, with bacterivores, fungivores, omnivores and predators in descending order of domination. Using nematodes as bioindicators of soil health hence suggests the poor level of soil health in the production areas surveyed in this study. Conclusively, results suggest that practicing monoculture as well as including highly susceptible summer crops such as soybean and sunflower in maize-based rotations are favourable to high population density build-ups of the predominant endo- and ectoparasitic nematodes identified. The findings of this study will assist nematologists and producers in making informed decisions to combat nematode problems in maize-based cropping systems.

Key words: Maize-based production, *Meloidogyne*, *Pratylenchus*, nematode abundance, beneficial nematodes.

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CHAPTER 1: GENERAL INTRODUCTION AND LITERATURE REVIEW

1.1 General introduction

The growing human population of South Africa (SA) is causing an increase in the demand for food (Jayne *et al.*, 2018), which results in challenges being faced in ensuring food security. In addition, grain crop production representing major food crops in developing countries (Ingram, 2011), is negatively affected by plant-parasitic nematodes, diseases, and pests (Fourie *et al.*, 2017; Miamoto *et al.*, 2017). The economically most important pests and diseases of grain that hamper production are listed in Table 1.3.

Nematode pests represent an important constraint to grain crop production worldwide and are responsible for substantial yield losses and poor-quality grains, particularly maize (Dababat and Fourie, 2018). Plant-parasitic nematodes cause approximately US\$157 billion in damages worldwide annually (Abd-Elgawad and Askary, 2015; Onkendi *et al.*, 2014). Crop fields that are heavily infested with plant-parasitic nematodes can experience yield losses of up to 80% with the severity of the damage being dependent on numerous abiotic and biotic factors (Moens *et al.*, 2009; Mao *et al.*, 2006). The most important genera of plant-parasitic nematodes that adversely affect grain production are root-knot (*Meloidogyne* Goeldi, 1892), lesion (*Pratylenchus* Filipjev, 1936) and cyst (*Heterodera* Ichinohe, 1952) nematodes (Dababat and Fourie, 2018; Lima *et al.*, 2015; Jones *et al.*, 2013).

According to Mc Donald *et al.* (2017), the most common root-knot nematodes species in SA that pose constraints to grain producers are *Meloidogyne incognita* (Kofoed and White, 1919) Chitwood, 1949 and *M. javanica* (Treub, 1885) Chitwood, 1949. Although *M. arenaria* (Neal, 1889) Cobb, 1890 is also present in local maize-based producing areas, the species have been recorded to a lesser extent than *M. incognita* and *M. javanica* (Pretorius, 2018; Visagie *et al.*, 2018). Moreover, in the Highveld Region of the Mpumalanga Province, another thermophilic species *M. enterolobii* Yang and Eisenback, 1983 was recently discovered in a maize-based production area (Pretorius, 2018; Visagie *et al.*, 2018). In terms of lesion nematodes, *Pratylenchus zeae* Graham, 1951 is the predominant lesion nematodes species that is abundant in local maize-based production areas (Mc Donald *et al.*, 2017; De Waele and Jordaan 1988; Walters, 1979a, b), followed by *P. brachyurus* (Godfrey, 1929) Filipjev and Schuurmans Stekhoven, 1941 (Mc Donald and De Waele 1987a, b; De Waele and Jordaan 1988). Other lesion nematode species associated with maize crop locally are *P. penetrans* (Cobb, 1917) Filipjev and Schuurmans Stekhoven, 1941; *P. crenatus* Loof, 1960; *P. delattrei* Luc, 1958; *P. neglectus* (Rensch, 1924) Filipjev and Schuurmans Stekhoven, 1941; *P. pratensis* (De Man, 1880) Filipjev, 1936; and *P. vulnus* Allen and Jensen, 1951 (Qiu *et al.*, 2016; Olowe, 2011; Hassan *et al.*, 2009; Bridge and Starr, 2007; Mc Donald and Nicol, 2005; Kleynhans *et al.*, 1996).

Although information exists regarding plant-parasitic nematodes associated with grain crops in SA (Pretorius, 2018; Mc Donald *et al.*, 2017), concerns by local grain producers and the industry initiated this study since the extent of damage caused by these invisible pests is still unknown (Mc Donald *et al.*, 2017). It is crucial that knowledge about the prevalence of economically important pests, such as plant-parasitic nematodes, be updated regularly so that producers know the identity and composition of the nematode communities prevailing in their fields. This way producers can properly plan crop rotation sequences and make informed decisions about managing plant-parasitic nematodes to limit yield losses and optimize income. Moreover, the discovery of new or known species that were not before recorded in a specific area is crucial and is dependent on continuous surveys. This study was therefore aimed at undertaking a nematode survey in 18 fields of grain crop producers in the Gauteng, North-West, and Free State provinces of SA to determine the predominant plant-parasitic nematodes species associated with the rhizospheres of the grain crops sampled. In addition, the project also focused on elucidating the status of beneficial nematodes to determine the health status of such soils using these organisms as bioindicators. Obtaining information on both plant-parasitic nematodes and beneficial nematodes can assist scientists in recommending management strategies to mitigate the damaging effects of economically important nematode pests. Moreover, knowledge generated about beneficial nematodes in grain fields will assist producers to realize how good or poor the health status of their soils is and guide them to make informed decisions on how to conserve this valuable resource to enable sustainable crop production and food security.

1.1.2 Research aims and objectives:

This study aims to determine the plant-parasitic and beneficial nematode assemblages in rhizospheres of grain crops in 18 fields of local, commercial producers.

Objectives:

- To determine the abundance and diversity of plant-parasitic nematodes (genus and/or species level) and beneficial nematodes (at family level) in each field sampled and their associations with selected abiotic parameters (rainfall, temperature, soil chemical and physical parameters).
- To identify root-knot nematode species from the 18 fields sampled using classical (perineal-pattern morphology) and molecular approaches.

1.1.3 Hypotheses:

Plant-parasitic nematode genera that will be recorded to dominate in grain crop rhizospheres are expected to be *Meloidogyne* and *Pratylenchus*, while it is expected that beneficial nematode abundance and diversity will be low in such intensified crop ecosystems. In addition, the

predominant *Meloidogyne* species foreseen to be identified are *Meloidogyne incognita* and *M. javanica*.

1.2 LITERATURE REVIEW

In SA, grain crops play a major role in the economy with an estimated 4.34 million hectares (ha) planted for summer grains during the 2020/21 production season, resulting in a 5% increase from the 2019/20 season (Sihlobo, 2021). The contribution of local grain crop production to the total gross local agricultural production, ranging between 25% and 33% (Beukes *et al.*, 2017), accentuates its importance in the economy sector. According to the Crop Estimates Committee of SA, these grain crops comprise maize (*Zea mays* L.), wheat (*Triticum aestivum* L.), soybean (*Glycine max* L.), sunflower (*Helianthus annuus* L.), barley (*Hordeum vulgare* L.), sorghum (*Sorghum bicolor* L.), oats (*Avena sativa* L.), dry bean (*Phaseolus vulgaris*) and canola (*Brassica napus* L.) (SAGIS, 2021) (Tables 1.1).

1.2.1 Production and importance of grain crops

During 2018, the agricultural sector in SA contributed 2.3% to the Gross Domestic Product (GDP), with a slight reduction to 2.2% in the second quarter of 2019 valued at ZAR74,158 billion (<https://www.bfap.co.za/wp-content/uploads/2023/04/Final-Baseline-2019.pdf>). The amount of land used for growing maize during the past 20 years was approximately 2 586 534 ha (Grain SA, 2021a) which is 50 % of SA's total land area (<https://www.GrainSA.co.za/pages/industry-reports/crop-estimates>). The majority of maize production is done by local commercial producers while developing farmers only cultivate maize on a small scale (Gouse *et al.*, 2016; Gouse, 2012) and are often associated with profitless, unproductive subsistence agriculture (<https://www.fao.org/3/w7365e/w7365e04.htm>). These latter producers traditionally practiced maize production in former homeland areas (*viz.*, Bophuthatswana, Ciskei, Gazankulu, KaNgwane, KwaNdebele, KwaZulu, Lebowa, QwaQwa, Transkei and Venda). Almost all field crop boundaries of local small-scale farms are situated in these former homeland areas of which a total of 650 000 ha (31%) is on high potential agricultural land. The areas planted for grain crops in SA and production figures for the preceding growing season of 2020/2021 are given in the tables below for summer and winter crops (Table 1.1). A concise summary of the production and importance of the grain crops sampled during this study, namely maize, sunflower, soybean and wheat, is given below.

Maize is an important cereal crop that forms part of the human diet and is also used as an important livestock feed source in large parts of the world (Adegbite, 2011). The crop is grown under a range of climatic conditions (Kagoda *et al.*, 2015) and is also, to a lesser extent, used for malting purposes and bioethanol production (SAGIS, 2021). Maize serves as the major staple food

source for some 200 million people in SA (Adisa *et al.*, 2018) and hence represents the biggest grain crop commodity grown by local producers, with white maize mainly cultivated for human consumption and yellow maize for animal fodder (SAGIS, 2021). The yields of local maize per ha range from 2,4 to 6,40 t/ha, and the 5,88 t/ha for 2021 yield is the second-largest yield produced since the 2016 and 2017 growing season of 6,40 t/ha (SAGIS, 2021).

Soybean is the No-1 legume crop grown in SA (Grain SA, 2021a) and one of the most important summer legume crops worldwide that serves as an important dietary protein and oil source for animal and human consumption but is also used for its medicinal purposes (Schmidt *et al.*, 2015; Liebenberg, 2012). Soybean seeds contain 19% – 23% oil and 30% – 40% protein (Schmidt *et al.*, 2015; Liebenberg, 2012). The production of soybean is extended to areas where predominately maize, groundnut, sunflower, potato, and wheat are traditionally grown (Fourie *et al.*, 2015). Hence, its wider production resulted in soybean being exposed to more biotic (pests and diseases) and abiotic (e.g., temperature, rainfall, daylight length) factors that have the potential to reduce local yields (Elhady *et al.*, 2018; Heinrichs and Muniappan, 2018; Fourie *et al.*, 2015; Liebenberg, 2012). Locally yields of 1,60 to 2,29 t/ha are realised nowadays, which are substantially higher than in the past (SAGIS, 2021).

Sunflower is the third most important oilseed crop produced in SA and can produce expected yields of 1,2 t/ha to 1,8 t/ha under drought conditions (Máali, 2017; DAFF, 2010); it is rated as the 4th most important vegetable oil seed sources after oil palm (*Elaeis guineensis* Jacq.), soybean, and rapeseed (*Brassica napus* subsp. *Napus* L.) (Máali, 2017). It is primarily used for manufacturing sunflower oil for human consumption and oil cake for animal feed because of its high protein content. Sunflower and soybean are important sources of oils and protein for both household and industrial purposes (Dlamini *et al.*, 2015), and sunflower oil can also be converted into biodiesel (ARC, 2018). Furthermore, the crop thrives under low-input farming conditions with little or no fertilizer generally being applied. Henceforth, sunflower is regarded as a low-input and beneficial cash crop following maize in SA (BFAP, 2015).

Wheat is the second most important staple food in SA (DAFF, 2016). The production of wheat for 2021 was 2,267 metric tons (MT) and the yield 4,31 t/ha, which was the fourth-highest yield ever recorded since the 2,504 MT obtained for the 2002 season (SAGIS, 2021; <https://www.statista.com/statistics/1135547/production-of-wheat-in-southafrica/#:~:text=In%202021%2C%20wheat%20production%20in%20South%20Africa%20was,highest%20amounting%20to%20about%202.5%20million%20metric%20tons>). Due to the low production of wheat locally, more than 40% of the domestic demand is met through imports. Hence, to prevent the looming food security crisis, production should be doubled by 2030 by advocating inclusion of the crop in maize-based rotations (BFAP, 2021).

Table 1.1: Area planted to and production of summer and winter grain crops in South Africa during 2021.

Crop	Area planted (ha)	Production (t)
Summer 2021		
White maize (<i>Zea mays</i> L.)	1 691 900	8 600 000
Yellow maize (<i>Zea mays</i> L.)	1 063 500	7 715 000
Total Maize (<i>Zea mays</i> L.)	2 755 400	16 315 000
Sunflower (<i>Helianthus annuus</i> L.)	477 800	677 240
Soybean (<i>Glycine max</i> (L.) Merr.)	827 100	1 897 000
Groundnut (<i>Arachis hypogaea</i> L.)	38 550	64 300
Sorghum (<i>Sorghum bicolor</i> (L.) Moench)	49 200	215 000
Dry bean (<i>Phaseolus vulgaris</i> L.)	47 390	57 672
Total	4 195 440	19 226 972
Winter 2021		
Wheat (<i>Triticum aestivum</i> L.)	523 500	2 257 205
Barley (<i>Hordeum vulgare</i> L.)	94 730	331 100
Canola (<i>Brassica napus</i> L.)	100 000	197 000
Oat (<i>Avena sativa</i> L.)	36 250	69 950
Total	754 480	2 855 255

Source: 1 CEC 10 February 2022 (<http://www.daff.gov.za/links/crop%20estimates>) or (<https://www.sagis.org.za/CEC-Feb-2022b.pdf>).

Key aspects about the production of these crops are summarized for background purposes in Table 1.2. Detailed information on the classification and biology of these crops is available in production guides such as those by Grain SA ([Graangids-2022 ENG-WEB.pdf available at sagrainmag.co.za](#)); Department of Agriculture, Forestry and Fisheries ([prodGuideSunflower.pdf](#) which is available at www.nda.agric.za/docs/Brochures/prodGuideSunflower.pdf) and the Agricultural Research Council ([ARC WINTER GUIDELINE 2022.pdf available at www.arc.agric.za/arc-sqi/Documents/PRODUCTION%20GUIDELINES/2022/ARC%20WINTER%20GUIDELINE%202022.pdf](#)).

Table 1.2: Important information on the areas and conditions under which the main grain crops in South Africa is produced

	Maize	Soybean	Sunflower	Wheat
Origin and distribution	Native to the Balsas River valley of southern Mexico and spread to the United States and Canada during the early 1500 BC. The crop was introduced to China, Brazil, France, Indonesia, Argentina and Africa, particularly South Africa in the 1655 (Sihlobo, 2018)	It was domesticated in the 1100 BC around the northeast of China and spread to tropical and subtropical regions, especially in South America, the Far East and Africa during the 1900 BC (DAFF, 2010)	Originated in North America during the 1000 BC. Wild sunflower Domesticated in the central United States (Liu and Burke, 2006). And introduced to Europe through Spain, and spread through Europe until it reached Russia and South Africa in 1860 (DAFF, 2010)	Originated in the Near East areas occupied by Syria, Turkey, Iran, Iraq Afghanistan, Iran and Iraq in Turkey during 6 500 BC (DAFF, 2016). Spread to India, Ethiopia, Ireland, Spain China and the Mediterranean countries in 1700 BC (Smith, 1995; DAFF, 2010). Introduced in the Cape province of SA in 1652 (DAFF, 2016: Smit <i>et al.</i> , 2010)
Taxonomic classification	Kingdom Plantae Class Liliopsida Order Poales Family Poaceae Genus <i>Zea</i> Species <i>Z. mays</i> L.	Kingdom Plantae Class Angiosperms Order Fabales Family Fabaceae Genus <i>Glycine</i> Species <i>G. max</i> (L.) Merr.	Kingdom Plantae Class Magnoliopsida Order Asterales Family Asteraceae Genus <i>Helianthus</i> Species <i>H. annuus</i> L.	Kingdom Plantae Class Liliopsida Order Poales Family Poaceae Genus <i>Triticum</i> Species <i>T. aestivum</i> L.
Growth stages	After planting the maize seed emerges after 6 days, and 2 leaves are fully emerged 7 days after. At 14 days after plating 5 leaves are fully developed and tassel and ear initiation. At 28 days after planting 8 leaves fully emerged and 42 days after planting 12 leaves are fully developed. At 56 days after planting 16 to 20 leaves are fully developed and tasselling or silking and pollination occurs at 66 days after planting. Maize plant reaches maturity at 70-100 days after planting (Du Plessis, 2003)	Vegetative stages (VE: Emergence; VC: Unrolled unifoliolate leaves; V1: first trifoliolate leaves V2: Second trifoliolate leaves; V3: third trifoliolate leaves); Reproductive stages (R1: Beginning flowering - plants have at least one flower on any node R2: Full flowering - there is an open flower at one of the two uppermost nodes; R3: Beginning pod - pods are 5 mm at one of the four uppermost nodes; R4: Full pod - pods are 2 cm at one of the four uppermost nodes and seed begin to develop which is 3 mm long in the pod at one of the four uppermost nodes on the main stem; R6: Full seed - pod containing a green seed that fills the pod capacity at one of the four uppermost nodes on the main stem; R7: Beginning maturity - one normal pod on the main stem has reached its mature pod colour; R8: Full maturity - 95% of the pods have reached their full mature colour)	Germination occurs 10 days after planting, leaves develop from 15 to 35 days after planting, flower bud 35-65 days after planting, and flowering stage occurs 65-83 days after planting. Seed development at 85-125 days after planting (DAFF, 2010)	After planting wheat seeds tillering occurs 10-30 days; stem elongation occurs 31-45 days after planting; heading and grain filling occurs 50-87 days after planting; at 93 days after plating harvesting occurs (DAFF, 2010; 2016)

Production areas, provinces	Free State (43,1%), North West (16,5%), Mpumalanga (24%), Gauteng (5%) and KwaZulu-Natal (5%) (SAGIS, 2021; Greyling and Pardey, 2019)	Free State (44.1%), Mpumalanga (35.1%), North West (8.4%), Gauteng (5.1%) and KwaZulu-Natal (4.4%) (SAGIS, 2021)	Free State (49.2%), North West (32.9%), Limpopo (16%), Gauteng (1%) (SAGIS, 2021)	Western Cape (69%), Free State (13%), Northern Cape (12%) (SAGIS, 2021)
Practices used for production	Traditionally conventional agriculture (CT), but conservation (CA) and regenerative agriculture (RA) practices gaining popularity	Traditionally conventional (CT) and conservation agriculture (CA)	Traditionally conventional agriculture (CT)	Conservation agriculture (CA) is increasing, but conventional agriculture (CT) still needed within the system
Cultivar choice (popularly used cultivars)	PAN 1623 RR; cv Dekalb 7677; cv PHI2370; DKC 73-76R; DKC75-65BR; DKC 76-75BR; MON76-75Bt; MON75-65Br; DKC 76-77BR, DKC 78-79Br; DKC6065Br; PAN 3R724	cv P64t39; cv Agriocare EC11; PHB6139RR; Pan 1523; Pan 1644	Pan7160 CLP; Pan 7080; Pan 7160	cv PANNAR 311
Optimal temperature	Seed germination: 10 °C; Optimum growth: 10 °C - 31 °C (Lizaso <i>et al.</i> , 2018; Du Plessis, 2003)	Seed germination: 12 °C; Optimum growth: 25 °C - 30 °C (DAFF, 2010)	Seed germination: 14 °C - 21 °C germination; Optimum growth: 23 °C - 34 °C (DAFF, 2010)	Optimum growth in cool areas 5 °C - 25 °C and warm areas 22 °C - 34 °C
Rainfall	600 mm - 900 mm	500 mm - 900 mm	500 mm -1000 mm	600 mm
Preferred soil type and pH	Sandy soils: <10% clay and clay-loam soils with clay content between 10 - 30 %. Soil pH (KCl) 5,0 - 5,5 (Du Plessis, 2003)	Sandy light texture soils and clay soils with 10% - 45% clay. Soil pH 5.8 - 6.5	Sandy loam and clay soils with 15 % - 55 % clay. Soil pH 5.2-6.1 (DAFF, 2010)	Loamy to sandy loam. Soil pH 6.0-7.5.5 and pH (KCl) <4.5 with acid saturation >8% (DAFF, 2016)
Fertilization	Mainly Nitrogen (N), Phosphorus (P), Potassium (K); N and K should not exceed 70 kg/ha, 50 kg/ha and 30 kg/ha for the respective row widths of 0.91, 1.5 and 2,1 or 2,3 m. Placed 70 to 100 mm next to and below the seeds (Du Plessis, 2003)	Mainly Nitrogen (N), Phosphorus (P), Potassium (K); 30 to 50 kg N, 40 kg P, requires large amount of K depending on the soil analysis. Row widths of 3 cm - 5 cm (2 cm in clay soil and 5 cm in sandy soil) (DAFF, 2010)	Low amounts or no fertilizer applied, but if applied it is mainly Nitrogen (N), Phosphorus (P), Potassium (K); 0 - 50 kg N/ha in clay soils; 0 - 70 kg N/ha in other soils, 0 – 21 kg P/ha depending on targeted yield; 50 kg/ha to 100 kg/ha of a 3:2:1 (25) (N.P.K) mixture. Row widths ranges from 90-100 cm, and wider rows of 1.5 - 2.1 m. (DAFF, 2010)	Mainly Nitrogen (N), Phosphorus (P), Potassium (K); N of 60 kg/ha - 140 kg/ha, P of 6-12 kg/ha, K of 15-40 kg/ha varies depending on the target yield and soil status. Row widths of 250 mm - 300 mm, with inter- and intra-row spacing of 40 cm - 50 cm and 7 cm – 15 cm, respectively (DAFF, 2016)
Planting time and depth	October-November in cooler eastern producing areas; in central areas October to mid-November; in drier western areas November to mid-December. Depth from 5 cm - 10 cm, depending on the soil type (Pretorius, 2018; DAFF, 2008; Du Plessis, 2003)	November and December and at depth of 10 to 15 cm (DAFF, 2010)	November- and December in eastern areas and mid-January in the western areas; at a depth of 25 mm, but in sandy soils up to 50 mm (DAFF, 2010)	Mid-April to mid-June in winter rainfall areas and mid-May to late July in summer rainfall; at a depth of 5 cm depending on the cultivar and growth period (DAFF, 2016)
Harvesting	Mechanically when seed moisture content is 12.5% - 14%	120 days - 130 days after planting or when seed moisture content is 15% - 20% (DAFF, 2010)	125 days after planting when seed moisture content is 10%	November/December; seed moisture content of 16%. A 13% seed moisture content is preferred for storage

Disease and weed control	Weeds are effectively controlled during the first 6 – 8 weeks after planting since it pollutes grain seeds resulting in downgrading of batches by transmitting odours to grain or incur additional costs to remove weed seeds. Seeds of certain weeds such as thorn apple (<i>Datura stramonium</i> L.) are poisonous when consumed by animals or humans. Weeds are also intermediate or alternative hosts of nematode pests another constraint to producers (Ntidi <i>et al.</i>, 2015). Glyphosate applications are mainly done due to majority of cultivars being glyphosate tolerant (Newman <i>et al.</i>, 2016; Dlamini <i>et al.</i>, 2014)	Weeds are mechanically controlled before planting while plants are 5 cm - 10 cm high. Removed manually after 6-8 weeks (DAFF, 2010). Glyphosate applications are mainly done due to majority of cultivars being glyphosate tolerant (Mbatyoti <i>et al.</i>, 2020: 2018; James, 2015; Dlamini <i>et al.</i>, 2014)	Weeds are controlled mechanically before the plant is tall. chemical control using grass and broadleaf herbicides (DAFF, 2010)	Non-selective herbicides are applied and are substituted by deep ploughing/tillage followed by harrow or sweep to bury the weeds and contaminated or diseased residues. For stubborn weeds, rotation with canola and/or pasture crops is advised to break the cycle
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1.2.2 Grain sectors and production practices

According to Van der Laan *et al.* (2017), the commonly used crop production systems have harmful impacts on the environment; this particularly refers to conventional agriculture (ConvA) systems. The ConvA practices are still the main approach used by SA producers (Swanepoel *et al.*, 2018) and are characterised by monocropping, various intensive soil tillage approaches (representing a major cost factor in grain production), over-use of synthetic agrochemicals, and inappropriate crop residue management (Sanaullah *et al.*, 2020; Van der Laan *et al.*, 2017). Tillage, still the most commonly used practice in local grain fields, mainly is aimed at controlling weeds, reducing water and wind erosion and incorporating organic matter from previous seasons' crop residues into the soil to improve the soil structure (Smit and Van den Berg, 2003). However, this approach can and does reduce the ability of soil ecosystems to regulate the occurrence of pests and diseases (Duru *et al.*, 2015) and usually leads to aggravated plant-parasitic nematodes infestations, especially root-knot nematodes, lesion nematodes, and other soil pathogens dominating in ConvA systems (Atandi *et al.*, 2017). These systems also promote global warming through the release of greenhouse gasses caused by intensive tillage practices, which further affect rapid organic matter (OM) decomposition leading to major soil carbon (C) reductions (Sanaullah *et al.*, 2020). Moreover, biodiversity loss, including that of beneficial soil nematodes (Tahat *et al.*, 2020), is an added disadvantage of monocropping and the over-use of inorganic fertilizers and pesticides (Mamabolo *et al.*, 2020). Agricultural soils in southern Africa are rapidly degrading as a result of the rate and manner of recent agricultural intensification (Kopittke *et al.*, 2019). It is hence not surprising that Du Preez and Van Huyssteen, (2020) estimated that 25% of SA's soils are already severely degraded as a result of anthropogenic activities related to agriculture and forestry.

In recent years, local farmers started to introduce more sustainable conservation (CA) practices, which are alternatives to ConvA, such as no-till or minimum till, maintenance of soil mulch cover (at least 30%) and diversification of crops (crop rotation) to improve soil, nutrient, and water management (Omulo *et al.*, 2022; Esmeraldo, 2017; FAO, 2014). These practices aim to conserve the health of soils and increase population levels and diversity of the beneficial soilborne organisms present. Increased abundance and diversity of beneficial organisms improve the soil quality and restore nutrients that were depleted through long-term conventional tillage and poor soil management of certain agricultural areas (Gotosa *et al.*, 2021; Grain SA, 2021b; FAO, 2008). Conservation agriculture ultimately targets the sustainable intensification of food production with a lower environmental impact (Swanepoel *et al.*, 2018).

Another practice that is gaining popularity nowadays in SA is regenerative agriculture (RA) representing agricultural management strategies similar to that of CA to promote soil health with livestock integration and cover crops growing all year round, as well as the addition of compost teas and manures being added to practices of CA (Giller *et al.*, 2021; Schreefel *et al.*, 2020; Soto *et al.*, 2020). The latter two approaches thus mainly focus on the use of cover crops in rotation, livestock integration, alternative nutrient management (through organic manures and composts), growing of stress-tolerant crop varieties, as well as the judicious and prudent use of synthetic agrochemicals (Thierfelder *et al.*, 2018; Walters *et al.*, 2016; Rusinamhodzi *et al.*, 2013; Owenya *et al.*, 2011). The ultimate aim is to improve microbiological diversity (Kriel, 2019) and is focused on optimizing soil health. Soil health refers to “the continued capacity of soil to function as a vital living system, within ecosystem and land-use boundaries, to sustain biological productivity, promote the quality of air and water environments, and maintain plant, animal, and human health” (Doran and Safley, 1997). These allow the restoration of key soil ecosystem functions and services (Van Es and Karlen, 2019).

1.2.3 Biotic constraints facing grain producers, with focus on plant-parasitic nematodes

Aside from a range of abiotic limitations that grain producers are facing, Sikora *et al.* (2018) reiterated that a range of diseases and pests represent major restrictions to producers in Sub-Saharan Africa and local producers with plant-parasitic nematodes being singled out as the ‘Big give away’. The presence and predictability of diseases and pests in maize-based production systems, however, vary because of the diverse climates and rainfall patterns that prevail in the different production areas (Sibiya *et al.*, 2013). Diseases and pests that are major constraints to SA grain producers (Sibiya *et al.*, 2013) and threaten grain crop production (with focus on maize, soybean, sunflower and wheat) are listed in Table 1.3 below.

Table 1.3: The most important diseases and pests that threaten grain crop production in South Africa with focus on maize, soybean, sunflower and wheat

	Maize	Soybean	Sunflower	Wheat
Diseases	Northern corn leaf blight (<i>Setosphaeria turcica</i>); Eyespot (<i>Aureobasidium zeae</i>); Phaeosphaeria leaf spot (<i>Phaeosphaeria maydis</i> (Henn.) Rane, Payak and Renfro); Rust (<i>Puccinia sorghi</i>); Grey leaf spot (<i>Cercospora zeae-maydis</i>); Maize streak virus (MSV); Ear rot (<i>Fusarium verticillioides</i> Tehon and Daniel); Damping off (<i>Rhizoctonia solani</i>) (Schoeman and Craven, 2013); Seedling blight (<i>Pythium</i> spp.); Charcoal Rot (<i>Macrophomina phaseolina</i>) (Schoeman and Craven, 2013) (Mc Donald et al., 2017; Sibiya et al., 2013; Varsani, et al., 2008; Bate and van Rensburg, 1992)	White mold (<i>Sclerotinia sclerotiorum</i>); Leaf blight (<i>Alternaria</i> spp.); Charcoal Rot (<i>Macrophomina phaseolina</i>); Sudden death syndrome (caused by various <i>Fusarium</i> spp.) (Hartman et al., 2009); Soybean rust (<i>Phakopsora pachyrhizi</i> and/or <i>P. meibomiae</i>) (Murithi et al., 2016; Jarvey, 2009; Fourie et al., 2001a); Leaf spot (<i>Cercospora soja</i>); Red leaf blotch (<i>Coniothyrium glycines</i>); Bacterial blight (<i>Pseudomonas savastanoi</i> pv. <i>glycinea</i>)	<i>Sclerotinia</i> head and stem rot (<i>Verticillium dahliae</i>); Sunflower rust (<i>Puccinia helianthi</i>) Schw. (Phe) (Ramusi and Flett, 2014); Leaf spot (<i>Septoria</i> and <i>Alternaria</i> spp.); Charcoal Rot (<i>Macrophomina phaseolina</i>); White Blister (<i>Pustula helianthicola</i>); Downy mildew (<i>Plasmopara halstedii</i>); Powdery mildew (<i>Erysiphe cichoracearum</i>); <i>Verticillium</i> wilt (<i>Sclerotinia sclerotiorum</i>); Phoma black stem (<i>Phoma macdonaldii</i>) (Fourie et al., 2017; Kleynhans et al., 1996)	Stem rusts (<i>Puccinia graminis</i> f. sp. <i>tritici</i>), leaf rust (<i>Puccinia triticina</i>); Stripe rust (<i>Puccinia striiformis</i> f. sp. <i>tritici</i>); Powdery mildew (<i>Erysiphe</i> [Blumeria] <i>graminis</i> f. sp. <i>tritici</i>); Maize streak virus (MSV), <i>Fusarium</i> head blight (<i>Fusarium graminearum</i> , Gert van Coller); Loose smut (<i>Ustilago tritici</i>); Karnal Bunt (<i>Tilletia indica</i>); <i>Fusarium</i> crown rot (<i>Fusarium pseudograminearum</i> , Sandra Lamprecht); Take-all (<i>Gaeumannomyces graminis</i> var. <i>tritici</i>); Septoria leaf blotch (<i>Septoria tritici</i>); Eyespot (<i>Helgardia herpotrichoides</i>) (ARC-SG, 2020)
Insects	Stalk borers (<i>Busseola fusca</i> , Fuller); The fall army worm (<i>Spodoptera frugiperda</i>) (Phophi et al., 2020); Weevils (<i>Sitophilus zeamais</i>); Black maize beetle (<i>Heteronychus arator</i>); African bollworm (<i>Helicoverpa armigera</i>) (Goergen et al., 2016; Bekker et al., 2016; Kleynhans et al., 1996)	Stem flies (<i>Melanagromyza sojae</i> and <i>Ophiomyia phaseoli</i>); American serpentine leaf miner (<i>Liriomyza trifolii</i>); African bollworm (<i>Helicoverpa armigera</i>); The bean leaf roller (<i>Omiodes diemenalis</i>); Soybean looper (<i>Thysanoplusia orichalcea</i>) (Hartman et al., 2009; Heinrichs and Muniappan, 2018; Ohnesorg and Hunt, 2015), legume pod borer (<i>Maruca vitrata</i>), the southern green stink bug (<i>Nezara viridula</i>); Soybean leaf miner, (<i>Aproaerema modicella</i> Deventer) (Fourie et al., 2015: 2001a; Du Plessis, 2001)	Weevils (<i>Cylindrocopturus adspersus</i>); American bollworm (<i>Heliothis armigera</i> (Hubner) Von Maltitz et al., 1993); Black maize beetle (<i>Heteronychus arator</i>); Astylus beetle (<i>Astylus atromaculatu</i>)	Russian wheat aphid (<i>Diuraphis noxia</i>); Bollworm (<i>Helicoverpa armigera</i>); Black maize beetle (<i>Heteronychus arator</i>); Leafhoppers (<i>Cicadulina mbila</i>); Brown wheat mite (<i>Petrobia latens</i>); False wireworm (<i>Somaticus</i> spp., <i>Gonocephalum</i> sp.); False army worm (<i>Leucania loreyi</i>) and leaf miners (<i>Agromyza ocularis</i>) (ARC-SG, 2020)

Plant-parasitic nematodes	<i>Meloidogyne</i> spp. Goeldi, 1892 [<i>M. arenaria</i> (Neal, 1889) Cobb, 1890, <i>M. incognita</i> (Kofoed and White, 1919) Chitwood, 1949, <i>M. javanica</i> Treub, 1885, <i>M. enterolobii</i> Yang and Eisenback, 1983]; <i>Pratylenchus</i> spp. Filipjev, 1936, <i>P. brachyurus</i> (Godfrey, 1929) Filipjev and Schuurmans Stekhoven, 1941, <i>P. crenatus</i> Loof, 1960, <i>P. delattrei</i> Luc, 1958; <i>P. neglectus</i> Rensch, 1924, <i>P. penetrans</i> Cobb, 1917, <i>P. pratensis</i> (De Man, 1880) Filipjev, 1936, <i>P. vulnus</i> Allen and Jensen, 1951, <i>P. zaeae</i> Graham, 1951]; <i>Rotylenchulus</i> Linford and Oliveira, 1940; <i>Scutellonema</i> Andrassy, 1958; <i>Hemicycliophora</i> de Man, 1921; <i>Helicotylenchus</i> Steiner, 1945; <i>Ditylenchus</i>; <i>Rotylenchus</i>; <i>Telotylenchus</i> Siddiqi, 1960; <i>Longidorus</i> Micoletzky, 1922; <i>Xiphinema</i> spp. Cobb, 1913; <i>Tylenchorhynchus</i> Cobb, 1913; <i>Quinisulcius</i>; <i>Trichodorus</i> spp. Cobb, 1913 and <i>Paratrichodorus</i> Siddiqi, 1974	<i>Meloidogyne arenaria</i>; <i>Meloidogyne ethiopica</i> Whitehead, 1968; <i>Meloidogyne hapla</i> Chitwood, 1949; <i>Meloidogyne incognita</i>; <i>Meloidogyne javanica</i>; <i>Pratylenchus brachyurus</i>; <i>Pratylenchus crenatus</i> Loof, 1960; <i>Pratylenchus flakkensis</i> Seinhorst, 1968; <i>Pratylenchus neglectus</i>; <i>Pratylenchus scribneri</i> Steiner, 1943; <i>Pratylenchus penetrans</i>; <i>Pratylenchus teres</i> Khan and Singh, 1975; <i>Pratylenchus thornei</i> Sher and Allen, 1953; <i>Pratylenchus zaeae</i>; <i>Pratylenchus vulnus</i>; <i>Criconemoides</i> Taylor, 1936; <i>Geocenamus</i> Thorne and Malek, 1968; <i>Helicotylenchus</i>; <i>Longidorus</i>; <i>Nanidorus</i> Siddiqi, 1974; <i>Radopholus</i> Thorne, 1949; <i>Scutellonema</i>; <i>Tylenchorhynchus</i> and <i>Xiphinema</i>	<i>Meloidogyne arenaria</i>, <i>M. hapla</i>, <i>M. incognita</i>, <i>M. javanica</i>; <i>Helicotylenchus</i>; <i>Longidorus</i>; <i>Nanidorus</i>; <i>Paratrophurus</i> Arias, 1970; <i>Pratylenchus</i>; <i>Quinisulcius</i> Siddiqi, 1971; <i>Rotylenchulus</i>; <i>Rotylenchus</i> Filipjev, 1936; <i>Scutellonema</i>; <i>Trophurus</i> Loof, 1956 and <i>Xiphinema</i> spp.	<i>Meloidogyne arenaria</i>, <i>M. chitwoodi</i> Golden, <i>M. incognita</i>, <i>M. javanica</i>; <i>Ditylenchus</i> Filipjev, 1936; <i>Pratylenchus brachyurus</i>; <i>Pratylenchus crenatus</i>; <i>Pratylenchus neglectus</i>; <i>Pratylenchus penetrans</i>; <i>Pratylenchus thornei</i>; <i>Pratylenchus zaeae</i>; <i>Rotylenchulus</i>; <i>Criconema</i> Hofmänner and Menzel, 1914; <i>Criconemoides</i>; <i>Dorylaimellus</i> Cobb, 1913; <i>Geocenamus</i>; <i>Helicotylenchus</i>; <i>Hemicycliophora</i>; <i>Longidorus</i>; <i>Paralongidorus</i> Siddiqi Hooper and Khan, 1963; <i>Paratylenchus</i> Micoletzky, 1922; <i>Pratylenchoides</i> Winslow, 1958; <i>Quinisulcius</i>; <i>Scutellonema</i>; <i>Tylenchorhynchus</i> spp and <i>Xiphinema</i> spp., <i>Rotylenchus</i>
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2 NEMATODES

Nematodes (Phylum Nematoda Potts, 1932), microscopic roundworms, are aquatic animals present in every ecological niche on earth (Decraemer and Hunt, 2013), and include both plant-parasitic nematodes and beneficial nematodes (Yeates *et al.*, 1993). The plant-parasitic nematodes infect and damage crop plants with their stylets (feeding structures located in the head), while beneficial nematodes are often used as bioindicators to indicate the health of soils since they play an important role in nutrient cycling and decomposition pathways (Neher, 2001; Bongers and Ferris, 1999).

Nematodes are classified into trophic groups, based on their feeding strategies. The main trophic groups consist of plant feeders (plant-parasitic nematodes or herbivores), bacterial feeders (bacterivores), fungal feeders (fungivores), omnivores, and predators (feeding on other micro-organisms), as well as animal and insect parasites which will not be focused on for this study (Sochová *et al.*, 2006; Neher, 2001; Bongers and Bongers, 1998).

2.1 Plant-parasitic nematodes

Plant-parasitic nematodes are included in the classes Enoplea Inglis, 1983 and Chromadorea Inglis, 1983, and represented by three orders namely Dorylaimida Pearse, 1942, Triplonchida Cobb, 1920 and Rhabditida Chitwood, 1933 (Decraemer and Hunt, 2013). These nematodes penetrate the cell walls of host tissues they infect with one of three types of stylets located in the anterior end (head) of their bodies., namely an onchiostylet (Triplonchida), odontostylet (Dorylaimida) and stomatostylet (Tylenchida, to which *Meloidogyne* belongs) (Van den Berg *et al.*, 2017).

Additionally, plant-parasitic nematodes are classified into different feeding groups, namely ectoparasites and endoparasites (Perry and Moens, 2011). Ectoparasitic nematodes remain in the rhizosphere during feeding, using their stylets to obtain food from the epidermal or outer root cortex cells. Endoparasitic nematodes on the other hand enter the root/other below-ground plant parts and feed, either completely or partially, inside the tissue. Migratory endoparasitic nematodes, such as *Pratylenchus* Filipjev, 1936, migrate inter-or intracellularly while feeding on root cortex cells of the host plant, causing damage (in the form of necrosis) to the plant along their migration pathway (Jones *et al.*, 2013). According to Gheysen and Mitchum (2011) endoparasitic, sedentary nematodes, however, display the most complex feeding approach by selecting cells in the vascular cylinder to be converted into a specialised feeding site, e.g. giant cells formed by *Meloidogyne* with the female becoming sedentary with the onset of feeding. However, the most important cyst nematodes *Heterodera avenae* Wollenweber, 1924, *H. zaeae* Koshy, Swarup, and Sethi, 1971 and *Punctodera chancoensis* Stone, Sosa Moss and Mulvey, 1976 are causing damage to maize in the Middle East (Egypt, Iran, Pakistan, Saudi Arabia, Syria, Turkey) and across Europe. Only *H.*

avenae has been found to date in SA and only infecting wheat of the main grain crops discussed in this chapter (Dababat and Fourie, 2018).

This study is primarily focused on plant-parasitic nematodes that occur in rhizospheres in maize-based production areas in SA, with emphasis on the genus *Meloidogyne* and to a lesser extent *Pratylenchus*. Thus, a concise summary of the economically most important plant-parasitic nematodes associated with grain crops in SA, namely symptoms, classification, identification (using classical and molecular approaches), other selected biological aspects (life cycle and reproduction strategies), and management of these two genera is given below. This is followed by selected information on beneficial nematodes (trophic groups, role as bioindicators, and faunal analysis).

2.1.1 Economically important plant-parasitic nematodes infecting grain crops

Amongst the wide range of plant-parasitic nematodes that infect and damage grain crops in SA, the main genera that threaten sustainable crop production are *Meloidogyne* and *Pratylenchus* (in order of predominance) (Fourie *et al.*, 2017; Mc Donald *et al.*, 2017). These authors summarized the economically most important species of these two genera associated with maize, soybean, sunflower and wheat being *M. incognita*, *M. javanica*, *M. arenaria* and *M. enterolobii* Yang and Eisenback, 1983. Locally, *Pratylenchus brachyurus* (Godfrey, 1929) Filipjev and Schuurmans Stekhoven, 1941 has been listed as the predominant lesion nematodes species associated with local maize crops, followed by *Pratylenchus zeae* Graham, 1951 and other species such as *P. crenatus* Loof, 1960; *P. delattrei* Luc, 1958; *P. fallax* Seinhorst, 1968; *P. neglectus* (Rensch, 1924) Filipjev and Schuurmans Stekhoven, 1941, *P. penetrans* (Cobb, 1917) Filipjev and Schuurmans Stekhoven, 1941, *P. pratensis* (de Man, 1880) Filipjev, 1936; *P. scribneri* Steiner, 1943; *P. teres* Khan and Singh, 1975; *P. thornei* Sher and Allen, 1953 and *P. vulnus* Allen and Jensen, 1951 (De Waele and Jordaan, 1988). Plant-parasitic nematodes cause considerable damage during crop development, which is intensified under drought and other stress conditions (Coyne *et al.*, 2009). Apart from root-knot nematodes and lesion nematodes, other plant-parasitic nematodes genera such as *Helicotylenchus*, *Longidorus*, *Nanidorus*, *Paratrophurus*, *Quinisulcius*, *Rotylenchulus*, *Rotylenchus*, *Scutellonema*, *Trophurus*, *Tylenchorhynchus*, and *Xiphinema* have been reported infecting grain crops in SA (Marais *et al.*, 2017; Bolton *et al.*, 1989; Keetch and Buckley 1984). However, damage caused by these genera is not documented due to their erratic occurrence in grain fields.

2.1.1.1 Root-knot nematodes (*Meloidogyne* spp.)

Root-knot nematodes are listed as the economically most important genus damaging crops worldwide (Jones *et al.*, 2013) and have a wide host range (Moens *et al.*, 2009), which explains its high pest status and the challenge to effectively control it. According to Ghaderi and Karssen

(2020), 105 *Meloidogyne* spp. have been identified and this number is likely to increase with the continuous discovery of new species especially with the use of highly effective molecular techniques. The predominant *Meloidogyne* spp. that damage maize crops in SA and account for up to 60% of yield losses are *M. incognita* (Kofoed and White, 1919) Chitwood, 1949 and *M. javanica* (Treub) Chitwood, 1949 and *M. arenaria* (Neal, 1889) Cobb, 1890 (Riekert and Henshaw, 1998; Kleynhans *et al.*, 1996; Riekert, 1996). Although *M. incognita* and *M. javanica* are listed as having the greatest damage potential in maize-production areas in SA (Mc Donald *et al.*, 2017), the recent discovery of *M. enterolobii* in grain production areas (Pretorius, 2018; Visagie *et al.*, 2018) should be noted since no pathogenicity studies have been done for this species under field conditions to date. Concerning soybean in SA, *M. incognita* and *M. javanica* are also the predominant species associated with the crop and have the potential to cause total yield loss (Fourie *et al.*, 2015). Other root-knot nematode species associated with soybean in SA are *Meloidogyne hapla* Chitwood, 1949, and *Meloidogyne ethiopica* Whitehead, 1968 (Engelbrecht *et al.*, 2020). According to Kleynhans *et al.* (1996), *M. incognita*, *M. arenaria* and *M. javanica* are the predominant parasites that cause damage to sunflower, while *M. hapla* has also been listed in association with this crop. The latter authors also listed *M. arenaria*; *Meloidogyne chitwoodi* Golden, O'Bannon, Santo and Finley, 1980, *M. incognita*; and *M. javanica* associated with wheat.

2.1.1.1.1 Symptoms of root-knot nematodes

Above-ground symptoms due to plant-parasitic nematode infection are mostly not specific to a genus and not recognised by producers but may include stunting, leaf chlorosis, wilting, and patchy growth (Fig. 2.1a). Mc Donald *et al.* (2017) mentioned that during extreme drought conditions, stunted maize plants infected with *Meloidogyne* spp., showed phosphate (PO_4^{3-}) deficiencies that were visible as purple discoloration of leaf edges. Above-ground symptoms are, however, difficult to distinguish from similar symptoms caused by other pests and/or diseases that concurrently infest maize plants or result from nutrient deficiencies or other abiotic factors (drought, excessive rainfall, and others).

Below-ground symptoms caused by root-knot nematodes usually consist of root galling, which may be distinct, and/or stunting or general poor development of roots. The size of the galls is generally determined by many factors such as the species, host status of the genotype infected and the number of infective second-stage juveniles (J2) (Karssen *et al.*, 2013). Hence, such abnormalities reduce the capability of the plant to translocate water and nutrients effectively from the roots/other below-ground parts to the aerial parts due to the formation of giant cells (Abad *et al.*, 2009). Also, in heavily infected maize roots, root-knot nematodes females may be visible where secondary roots emerge from the primary roots (Fig. 2.1b) (Mc Donald *et al.*, 2017). In severe cases of root-knot nematode infection, the survival of the host plant and crop yield/quantity is compromised (Abad *et al.*, 2009).

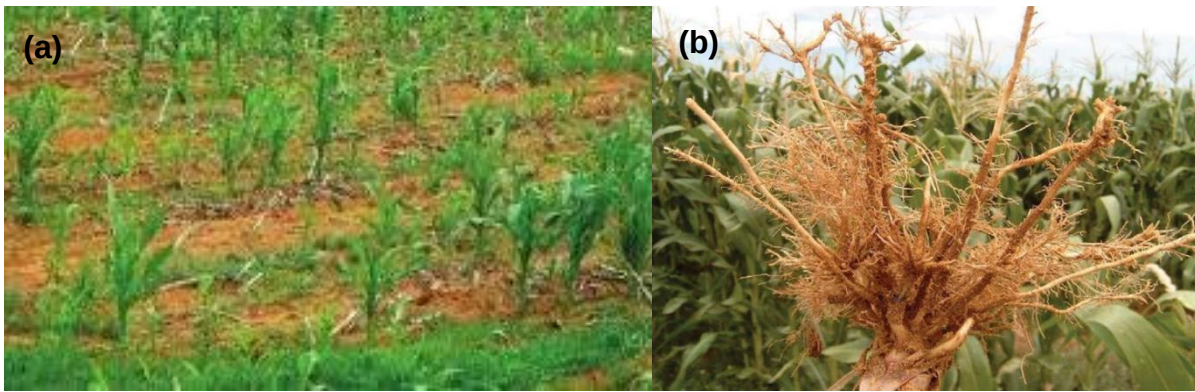


Figure 2.1a & b: (a) Above-ground damage caused by root-knot nematode infection showing poor seedling emergence and stunted maize plants (Photo: Chris Schmidt: Sidi Parani, SA); (b) Below-ground damage of a maize root covered with inconspicuous galling and branching caused by *Meloidogyne* infection (Photo: A.H. Mc Donald, North-West University, SA).

In soybean and sunflower fields, above-ground symptoms of *Meloidogyne* spp. infection are usually visible as stunted plants with leaf chlorosis (Bridge and Starr 2007; Sikora *et al.*, 2005) and patches of poor growth (Figs. 2.2 and 2.3); similar to those evident in maize fields. Below-ground parasitism is similar to galling of roots, as evident for maize (Bridge and Starr 2007), but it is important to consider that on soybean roots, *Meloidogyne* spp. root galls can be mistaken for *Rhizobium* spp. nodules that are vital for nitrogen fixing by the plant (Liebenberg, 2012).

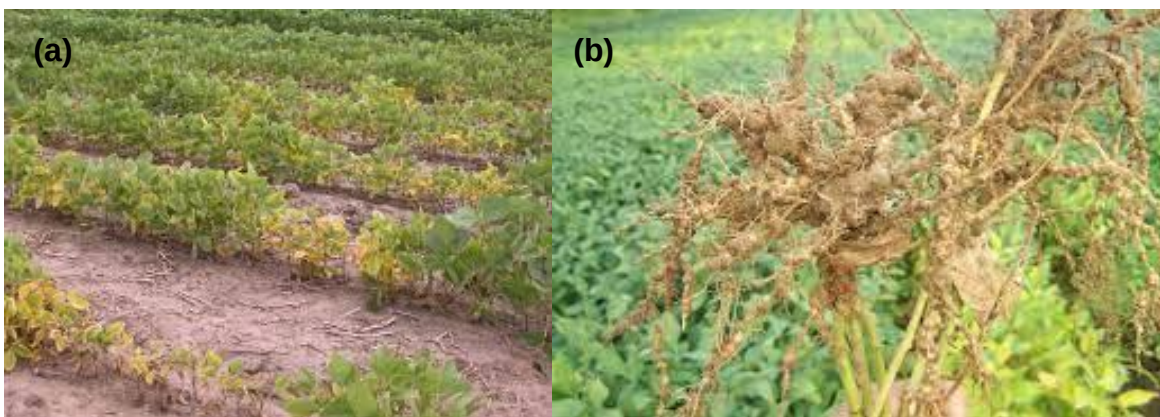


Figure 2.2a & b: (a) Stunted growth and yellowing of soybean plants infected with root-knot nematodes (b) Heavily galled root systems of infected soybean plants that grew in root-knot nematode infested fields in the Viljoenskroon area (Free State province of SA) (Photo: Driekie Fourie, North-West University, SA).

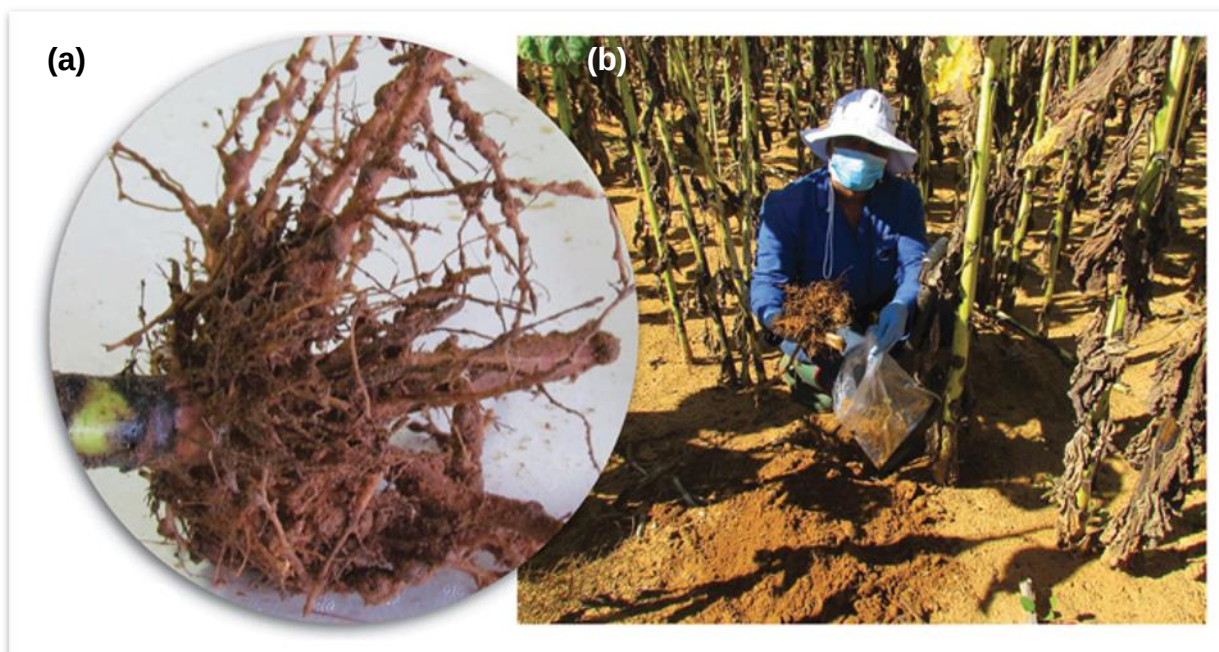


Figure 2.3a & b: (a) Root-knot nematode damage on infected sunflower roots showing root galls and (b) sampling of roots for nematode analysis (Photo: Nancy Ntidi, ARC-Grain Crops, South Africa).

For wheat, above-ground symptoms resulting from root-knot nematodes infection include uneven patches of poorly growing or stunted plants that may vary in size from a few square meters to larger areas and which are randomly distributed throughout a field, as well as yellowing plants (Ibrahim *et al.*, 1999). Severely infected plants remain stunted, and their leaves become pale yellowish-green in colour, with thin narrow leaf blades (Fig. 2.4a) (Smiley, 2015; Castillo and Vovlas, 2007). Moreover, if ears are formed by such infected plants, they contain very few grains. *Meloidogyne*-infected plant roots show root galls, especially on the root tips, which are typically curved, horseshoe or spiral-shaped (Fig. 2.4b) (Dababat and Fourie, 2018).



Figure 2.4a & b: (a) Above-ground symptoms of root-knot nematode damage on wheat plants, with stunted growth and patches of better growing plants visible in a field (Photo: Alex Gwynne, Queensland, Australia) and (b) severe root galling caused by *Meloidogyne naasi* (Photo: D. Coyne, IITA, Kenya).

2.1.2 Lesion nematodes (*Pratylenchus* spp.)

Lesion nematodes are regarded as the second-most economically important plant-parasitic nematodes genus that infect and damage crops worldwide (Jones *et al.*, 2013), with generally the same applying for grain crops in SA (Fourie *et al.*, 2017; Mc Donald *et al.*, 2017). This genus is widely distributed throughout temperate and tropical environments, and they parasitise a wide variety of plant hosts, similar to root-knot nematodes. *Pratylenchus* comprises of many species that parasitise grain crops (Dababat and Fourie, 2018). Locally *Pratylenchus neglectus* is the most abundant species identified in wheat production areas (Mc Donald *et al.*, 2017) followed by *P. brachyurus*, *P. crenatus* and *P. zaei*. Other lesion nematodes that affect wheat are *P. penetrans* and *P. thornei* (Mc Donald *et al.*, 2017; Marais, 2006; Kleynhans *et al.*, 1996).

2.1.2.1 Symptoms of lesion nematodes

Above-ground symptoms caused by lesion nematodes in maize, soybean, sunflower, and wheat fields generally resemble those caused by other plant-parasitic nematodes, e.g., root-knot nematodes, with infected plants appearing dwarfed/stunted, patchy/irregular spots of poor-growing plants, premature yellowing of older leaves, with other growth parameters also being negatively affected (Dababat and Fourie, 2018; Fourie *et al.*, 2017; Jackson-Ziems, 2016; Jones *et al.*, 2013; Smiley, 2015; Smiley *et al.*, 2004).

Below-ground symptoms resulting from lesion nematodes infection according to Coyne *et al.* (2009) resemble visible brown/black lesions on maize roots, girdled young roots and pruned lateral

roots (Fig. 2.5). *Pratylenchus*-infected soybean plants show brown, necrotic areas on roots and a smaller root system compared to non-infected roots (Bridge and Starr, 2007; Sikora *et al.*, 2005) (Fig. 2.6a). Pods of heavily infected plants may also be smaller and only partly filled compared to those of uninfected plants (Engelbrecht *et al.*, 2020; Fourie *et al.*, 2017; Jones *et al.*, 2013) (Fig. 2.6b). According to De Waele and Elsen (2002) initial lesion nematodes damage in wheat roots appears as water-soaked lesions in the epidermis. Dead cortical and epidermal cells (appearing as brownish or blackish lesioned roots), loss of lateral roots and root hairs of infected wheat plants are visible because of lesion nematodes infection (Vanstone *et al.*, 1998) (Fig. 2.7). Lesion-like symptoms are, however, difficult to identify as those caused by lesion nematodes when other pests and/or diseases are present (Mc Donald and Nicol, 2005).



Figure 2.5: A maize root infected with lesion nematodes, resulting in brownish to blackish necrotic tissue (Photo: Danny Coyne, IITA, Kenya).



Figure 2.6: (a) Necrotic soybean roots infected with lesion nematodes, and (b) a stunted plant with a reduced and necrotic root system (green circle) compared to a noninfected plant (Photos: (a) Suria Bekker and (b) Driekie Fourie, North-West University, Potchefstroom, South Africa).

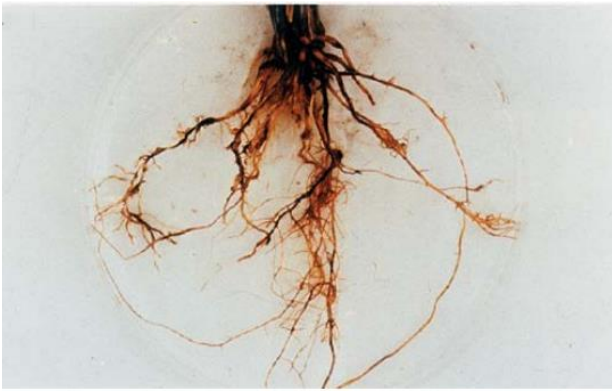


Figure 2.7: Symptoms of root lesion nematodes, *Pratylenchus thornei*, infection of wheat roots, showing extensive lesions, cortical degradation and reduction in both seminal and lateral root systems (Photo: by J. Nicol, CIMMYT).

Often, penetration of roots by lesion nematodes facilitates colonization by other pathogens, e.g., root-rotting fungi and bacteria resulting in increased yield losses (Smiley, 2015; Moens and Perry, 2009; Evans and Haydock, 1993). Table 1.5 lists the pathogenic microbes that are associated with lesion nematodes and root-knot nematodes infection in maize, soybean, sunflower and wheat.

Table 1.4: Microbial pathogens associated with *Meloidogyne* and *Pratylenchus* in maize, soybean, sunflower and wheat rhizospheres.

<i>Meloidogyne</i>			
Maize	Soybean	Sunflower	Wheat
<i>Fusarium</i> spp.; <i>Phytophthora</i> ; <i>Verticillium</i> ; <i>Rhizoctonia</i> (Chang <i>et al.</i> , 2018; Schoeman and Craven, 2013; Taylor and Sasser, 1978)	<i>Meloidogyne incognita</i> : <i>Ralstonia solanacearum</i> (bacterial wilt) (Furusawa <i>et al.</i> , 2019); <i>Rhizoctonia solani</i> (seedling blight) (Chang <i>et al.</i> , 2018; Back <i>et al.</i> , 2002); <i>Fusarium graminearum</i> , <i>F. equiseti</i> ; (Hartman <i>et al.</i> , 2019; Goswami and Agarwal, 1978); <i>F. brasiliense</i> (soybean sudden death syndrome) (Tewoldemedhin <i>et al.</i> , 2017)	<i>Ralstonia solanacearum</i> (causing bacterial wilt) (Peeters <i>et al.</i> , 2013); <i>Rhizoctonia solani</i> (seedling blight)] <i>Fusarium</i> ; <i>Phytophthora</i> <i>Verticillium</i> (Furusawa <i>et al.</i> , 2019; Chang <i>et al.</i> , 2018; Back <i>et al.</i> , 2002; Taylor and Sasser, 1978)	<i>Gaeumannomyces</i> ; <i>Rhizoctonia solani</i> (Foley <i>et al.</i> , 2016; Coombs <i>et al.</i> , 2004; Parmeela and Johri, 2004)
<i>Pratylenchus</i>			
<i>Fusarium moniliforme</i> (Jordaan <i>et al.</i> , 1987); <i>Fusarium verticillioides</i> (sacc) Nirenberg (Jordaan <i>et al.</i> , 1987); <i>Fusarium graminearum</i> ; <i>Verticillium</i> ; <i>Pythium</i> ; <i>Rhizoctonia</i> ; <i>Phytophthora</i> (Chang <i>et al.</i> , 2018; Taylor and Sasser, 1978)	<i>Rhizoctonia solani</i> (Liu <i>et al.</i> , 2016); <i>Fusarium</i> (Hartman <i>et al.</i> , 2019); <i>F. brasiliense</i> (soybean sudden death syndrome) (Tewoldemedhin <i>et al.</i> , 2017)	<i>Verticillium</i> ; <i>Pythium</i> ; <i>Sclerotinia sclerotiorum</i> (Furusawa <i>et al.</i> , 2019; Chang <i>et al.</i> , 2018; Back <i>et al.</i> , 2002; Taylor and Sasser, 1978)	Mycorrhizal colonization; <i>Rhizoctonia solani</i> (Foley <i>et al.</i> , 2016; Coombs <i>et al.</i> , 2004; Parmeela and Johri, 2004)

2.2 Sampling, identification and classification of nematodes, with focus on plant-parasitic nematodes.

2.2.1 Sampling and extraction

Ferris *et al.* (1981) stated that obtaining an adequate and representative soil sample is the first step in gathering high-quality data on nematode assemblages. The authors further mention that one could determine the exact number of nematodes in a field by removing all the soil and extracting all the nematodes. However, since cost and practicality impede such an undertaking, a compromise is to take samples in a representative way and to assume that the identity and abundance of nematode communities in the samples represent the region. The greater the proportion of the area sampled, the more accurately the nematode data to be generated.

There are no rigid criteria for determining the number of samples in relation to the size of the field, but besides cost and time factors, relevant considerations are:

- a) Soil heterogeneity or field stratification: The area should be stratified into regions of similar characteristics of soil texture, moisture, organic matter content, salinity, differences in cropping history, irrigation practices, etc., and samples representatively taken from each region.
- b) Seasonality: Conditions and resources in the soil differ with climatic changes and crop phenology. In ecology studies, soil sampling is typically undertaken when soil conditions are conducive to the biological activity of the target organisms. Sampling for plant-parasitic nematodes is often based on the cropping schedule, e.g., before planting to determine management needs or at the end of the crop season to determine nematode abundances and the effectiveness of the management. Moreover, assessing the nematode abundance and diversity at the end or near the end of a growing season is a valuable indicator for the producer to plan the successive crop to be grown on the field.
- c) Sample depth: Zones of expected biological activity in the soil should be considered. For instance, a greater abundance of plant-parasitic nematodes may be associated with feeder roots, while bacterial- and fungal-feeding nematodes may be associated with surface applications of organic material. Frequently, soil nematodes are distributed from the soil surface to the rooting depth of the current plant species.

With the above criteria in mind, four general sampling strategies can be followed (Tan *et al.*, 2005):

- a) Simple random sampling: In fields where, homogeneous conditions uniformly distributed are randomly selected (Bhardwaj, 2019; Rahi, 2017) (e.g., no stratification in terms of soil colour, texture, type and nutrients), this represents a valid and cost-efficient method to collect samples at random locations throughout the field.
- b) Systematic sampling: Fields with homogeneous conditions allow for systematic samples at regular intervals to ensure that all fields are equally embodied by the sampling.
- c) Stratified sampling: This method is usually employed in heterogeneous fields. Each field is segregated into various homogenous subgroups or strata, and a sample is obtained from each

and all strata are sampled separately. The number of samples collected in each stratum should be proportional to its size in relation to the whole area. Within each stratum, samples may be collected at random or systematically (<https://www.wallstreetmojo.com/stratified-sampling/>).

- d) Compositing: This method is widely used in survey studies undertaken in SA (for example Mbatyoti *et al.*, 2020; Rashidifard *et al.*, 2019b, c; Visagie *et al.*, 2018; Fourie *et al.*, 2001a) during which the sample units, e.g., soil cores or soil obtained by using a spade, are aggregated into a single sample. The composite sample should be mixed, thoroughly to obtain a representative subsample used for analysis. Although composite samples allow a greater proportion of the area to be sampled without increasing analysis and nematode identification costs, they do so at the cost of losing information on the distribution and variation in abundance of nematode populations that would be revealed by processing each sample unit individually.

Soil samples along with the corresponding plant roots sampled are placed inside a transparent plastic bag and labeled according to the number of samples, including a date sampled with the locality and/or farm. The samples are placed in a cooler box to conserve moisture and transported to the laboratory where it is stored at a temperature range of 6 °C to 11 °C for a maximum of 4-5 days. Such samples are prepared for extraction and then the basic methods used for extraction are applied (Kleynhans *et al.*, 1996) (see Paragraph 2.2.1.1 - 2.2.1.3) (maize: Pretorius, 2018; Agenbag, 2016, soybean: Mbatyoti *et al.*, 2020; Fourie *et al.*, 2001a, sunflower: Steenkamp *et al.*, 2015 and wheat: Steenkamp *et al.*, 2015).

2.2.2 Identification and classification

Accurate classification of *Meloidogyne* and *Pratylenchus* species is very important to make sure that correct management strategies are applied as well as for other purposes such as quarantine (Coyne *et al.*, 2007). As summarized above (Paragraph 2.2.1), accurate sampling, handling, and extraction of the plant-parasitic nematodes from rhizosphere samples should hence be followed to ensure that different life stages of the target species are available for identification (Marais *et al.*, 2017). The classification of *Meloidogyne* and *Pratylenchus* is given below in Table 1.5.

Table 1.5: Simplified outline classification of *Meloidogyne* and *Pratylenchus* according to the classification systems of Hunt and Handoo (2009) and Van den Berg *et al.* (2017).

Phylum Nematoda (Rudolphi, 1808) Lankester, 1877

Class Secernentea von Linstow, 1905

Subclass Diplogasteria Maggenti, 1982

Order Tylenchida Thorne, 1949

Suborder Tylenchina Chitwood, 1950

Superfamily Tylenchoidea Örley, 1880

Family Heteroderidae Filipjev and Schuurmans Stekhoven, 1941

Family Pratylenchidae Thorne, 1949

Subfamily Meloidogyninae Skarbilovich, 1959

Subfamily Pratylenchinae Thorne, 1949

Genus *Meloidogyne* Goeldi, 1892

Genus *Pratylenchus* Filipjev, 1936

2.2.3 Identification

Both morphology and morphometrics (also referred to as the classic approach), and molecular approaches (also referred to as new-generation techniques) should be and generally are followed nowadays to identify plant-parasitic nematodes species such as those of *Meloidogyne* and *Pratylenchus* (Marais *et al.*, 2017). The techniques used to discriminate among species of these two economically important plant-parasitic nematode genera are summarised in Table 1.6 (morphology and morphometrics) and Table 1.7 (molecular) below. To identify a nematode to species level, one must have in-depth knowledge and substantial experience of key characteristics that will indicate the species studied (Van Bezooijen, 2006; Kleynhans *et al.*, 1996). Morphological identification of plant-parasitic nematodes to species level is considered a highly specialised field of expertise, due to the numerous characteristics that must be studied and the overlapping of characteristics among species of the same genus (Collett, 2020; Rashidifard *et al.*, 2019b; Marais *et al.*, 2017). Hence, to ensure that key characteristics are used for the identification of a plant-parasitic nematodes species, well-prepared and preserved specimens must be a priority using well established and globally accepted protocols (Figs. 2.8, 2.9 & 2.10) (Marais *et al.*, 2017).

Table 1.6: Morphological characteristics and morphometric data used to identify *Meloidogyne* and *Pratylenchus* species.

<i>Meloidogyne</i>	Perineal-patterns of females	Oesophageal characteristics of females	Morphometrics of vermiform second-stage juveniles (J2) and males
	<i>M. arenaria</i> : Circular to oval with low dorsal arch to medium high and broadly rounded/squarish apex, short vertical lines may form along one/both lateral fields, around and outside phasmids (Collett, 2020; Rashidifard, 2019; Rashidifard <i>et al.</i> , 2019b; Agenbag, 2016; Karssen <i>et al.</i> , 2013; Brito <i>et al.</i> , 2004; Kleynhans, 1991; Eisenback, 1985; Yang and Eisenback, 1983)	Cylindrical or expands, narrows usually ovoid, occasionally spheroid metacarpus lining (Collett, 2020; Rashidifard, 2019; Rashidifard <i>et al.</i> , 2019b; Agenbag, 2016; Karssen <i>et al.</i> , 2013; Brito <i>et al.</i> , 2004; Kleynhans, 1991; Eisenback, 1985; Yang and Eisenback, 1983)	Body characters are generally used for characterizing species are body length; body width, dorsal oesophageal gland orifice (DEGO); stylet length; tail length; hyaline tail length (only J2) (Hunt and Handoo, 2009; Kleynhans, 1991); 'a' = total body length/total body width; 'c' = total body length/tail length (https://doi.org/10.5423/PPJ.OA.05.2018.0081) (see Fig. 2.9)
	<i>M. enterolobii</i> : round to ovoid with moderate high dorsal arch to high and rounded apex though square-like in some specimens along with distinct phasmids in the tail-terminus, fine striae and smooth pattern ventral side that are non-present in peri-vulval (Collett, 2020; Rashidifard, 2019; Rashidifard <i>et al.</i> , 2019b; Agenbag, 2016; Karssen <i>et al.</i> , 2013; Brito <i>et al.</i> , 2004; Kleynhans, 1991; Eisenback, 1985; Yang and Eisenback, 1983)	No information recorded for the lumen of oesophagus form/shape in pro- and meta-carpus (Collett, 2020; Rashidifard, 2019; Rashidifard <i>et al.</i> , 2019b; Agenbag, 2016; Karssen <i>et al.</i> , 2013; Brito <i>et al.</i> , 2004; Kleynhans, 1991; Eisenback, 1985; Yang and Eisenback, 1983)	
	<i>M. incognita</i> : Circular to oval with medium-high dorsal arch to high and broadly rounded or squarish apex with slanted phasmids and clear tail terminus along with smooth, wavy,	Lumen lining expands then narrows towards spheroid and visible as an ovoid metacarpus lining (Collett, 2020; Rashidifard, 2019; Rashidifard <i>et al.</i> ,	

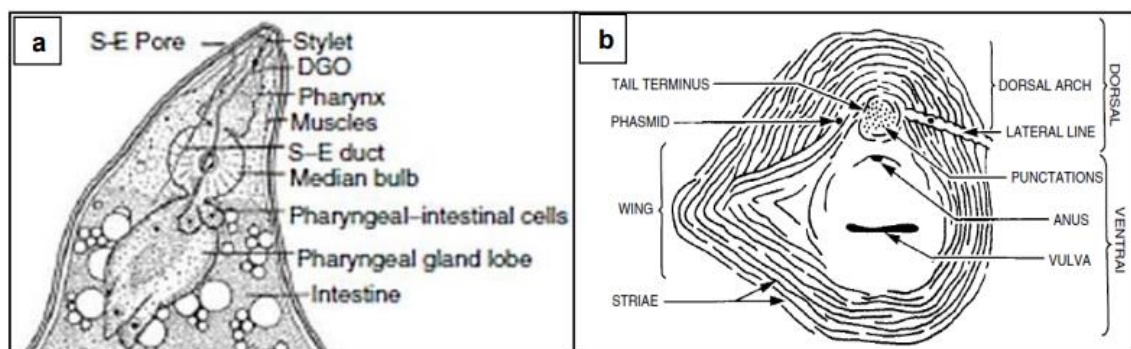
	and even zigzag striae pattern (Collett, 2020; Rashidifard, 2019; Rashidifard <i>et al.</i> , 2019b; Agenbag, 2016; Karssen <i>et al.</i> , 2013; Brito <i>et al.</i> , 2004; Kleynhans, 1991; Eisenback, 1985; Yang and Eisenback, 1983)	2019b; Agenbag, 2016; Karssen <i>et al.</i> , 2013; Brito <i>et al.</i> , 2004; Kleynhans, 1991; Eisenback, 1985; Yang and Eisenback, 1983)	
	<i>M. javanica</i> : Circular to oval with low dorsal arch to medium-high and squarish to broadly round apex; plasmids not visible with clear double lateral lines and rectal punctuations; smooth to wavy striae (Collett, 2020; Rashidifard, 2019; Rashidifard <i>et al.</i> , 2019b; Agenbag, 2016; Karssen <i>et al.</i> , 2013; Brito <i>et al.</i> , 2004; Kleynhans, 1991; Eisenback, 1985; Yang and Eisenback, 1983)	Procorpus lining cylindrical and may expand/narrow immediately in front of usually ovoid, occasionally spheroid metacarpus lining (Collett, 2020; Rashidifard, 2019; Rashidifard <i>et al.</i> , 2019b; Agenbag, 2016; Karssen <i>et al.</i> , 2013; Brito <i>et al.</i> , 2004; Kleynhans, 1991; Eisenback, 1985; Yang and Eisenback, 1983)	
<i>Pratylenchus</i>	Popular morphological characteristics used	Morphometrics of all vermiform life stages	
	Stylet length; Dorsal gland orifice (DGO); Maximum body length; Excretory pore; Pharynx length; Pharyngeal overlap; Ovary; Post-uterine sac; Tail; Vulva to anus distance; Lip diam; lip height (Loof, 1960)	Body characters are generally used for characterizing species are: stylet length; stylet bulb diameter; overall body length; body diameter; anterior end to vulva; tail size; body diameter at anus height; anterior end to the end of the oesophagus; anterior end to oesophagus-intestine junction; body diameter stylet, body length/largest body width; 'a' = total body length/anterior end to end of oesophagus; 'c' = total body length/tail length; anterior end to vulva/body length*100; body length/anterior end to oesophagus-intestine junction (https://doi.org/10.1590/1983-21252020v33n303rc) (see Fig. 2.10)	

Table 1.7: Biochemical and molecular techniques used to identify *Meloidogyne* and *Pratylenchus* species.

Techniques	<i>Meloidogyne</i>	<i>Pratylenchus</i>	Application in South Africa	Benefits	Drawbacks
Biochemical: Isozyme (esterase)	Yes. Blok and Powers, 2009; Carneiro and Almeida, 2001.	Yes. Ibrahim <i>et al.</i> (1995)	Not yet	Stability of the isozyme phenotypes within the mature female individuals	Only adult females can be used for identification as isozyme phenotypes are only expressed in this life stage
Sequence characterised amplified region (SCAR-PCR)	Yes. Tigano <i>et al.</i> , 2010; Randig <i>et al.</i> , 2002; Zijlstra <i>et al.</i> , 2000.	Yes. Waeyenberge <i>et al.</i> , 2000	<i>Meloidogyne</i> : [Buys <i>et al.</i> , 2022; Engelbrecht <i>et al.</i> , 2022; Collett, 2020; Girgan <i>et al.</i> , 2020a; Matlala <i>et al.</i> , 2020; Rashidifard <i>et al.</i> , 2019c; Rashidifard <i>et al.</i> , 2018; Visagie <i>et al.</i> , 2018; Fourie <i>et al.</i> , 2017; Agenbag, 2016; Jansen-Girgan <i>et al.</i> , 2016; Ntidi, 2016; Onkendi and Moleleki, 2013a; Mienie <i>et al.</i> , 2002; Fourie <i>et al.</i> , 2001a] <i>Pratylenchus</i> : [Visagie <i>et al.</i> , 2018; Knoetze <i>et al.</i> , 2017; Marais <i>et al.</i> , 2017]	Precise technique to differentiate between root-knot nematode species with low genetic differences according to DNA fragments length. Also identify multiple species in one PCR test	Number of primer pairs and gel electrophoresis (GE) bands that can be used in a single reaction without locating false positive results
Restriction fragment length polymorphism (RFLP)	Yes. Fargette <i>et al.</i> , 2005; Carpenter <i>et al.</i> , 1992; Curran <i>et al.</i> , 1985;	Yes. Waeyenberge <i>et al.</i> , 2000; Williams and Clair, 1993	Hallman and Subbotin, 2018; Blok and Powers, 2009; Berry <i>et al.</i> , 2008	Comparing new RFLP profiles to existing ones is a quick method of identification	Very time consuming and labour intensive. Cannot be used for mixed communities
Random amplified polymorphic DNA (RAPD)	Yes. Tigano <i>et al.</i> , 2010; Cenis, 1993	Yes	Onkendi and Moleleki, 2013b; Berry <i>et al.</i> , 2008	Allowed the development of a SCAR marker as a new tool (species-specific DNA)	Poor reproducibility
Quantitative PCR (qPCR)	Yes. Holterman <i>et al.</i> , 2012; Lopez-Nicora <i>et</i>	Yes Mokrini <i>et al.</i> , 2013	Onkendi <i>et al.</i> , 2014; Onkendi, 2012; Berry <i>et al.</i> , 2008	Provide fast and high throughput detection and quantification of target DNA	Requires a prior sequence data for desired target gene

	<i>al.</i> , 2012; Bates <i>et al.</i> , 2002			sequences in different matrices	
Amplified fragment length polymorphism (AFLP)	Yes. Fargette <i>et al.</i> , 2005; Suazo and Hall, 1999;	Yes	Hallman and Subbotin, 2018	Results are highly reproducible	Labour intensive and development cost is high
Recombinase polymerase amplification (RPA)	Yes	Yes	Ju <i>et al.</i> , 2019; Subbotin, 2019	Efficient and fast alternative for PCR. Can be carried out in real time. Versatile	Very little supporting literature. RPA specific primers have only been developed for <i>M. enterolobii</i> so far
Cytochrome oxidase subunit 1 (COI)	Yes. Derycke <i>et al.</i> , 2010; Blok, 2005	Yes	Moore <i>et al.</i> , 2020; ; Rashidifard <i>et al.</i> , 2019c; Subbotin, 2019; Powers <i>et al.</i> , 2018; Pretorius, 2018; Powers <i>et al.</i> , 2005; Blok <i>et al.</i> , 2002; Powers and Harris, 1993	Primer sets are usually genus specific. Aid in the discovery of previously undescribed species	Limited scope for use in taxonomy. Requires well-curated sequence databases to be accurate. Combination with other techniques is crucial for accuracy
Cytochrome oxidase subunit 2 (COII)	Yes. Powers and Harris, 1993	Yes	Rashidifard <i>et al.</i> , 2019c; Pretorius, 2018; Onkendi and Moleleki, 2013a, b	Overcomes limitations of rDNA techniques, due to mtDNA genes having uniparental inheritance and high mutation rates.	COII regions have insufficient variability to reliably differentiate between <i>M. incognita</i> , <i>M. javanica</i> and <i>M. arenaria</i> .
Nicotinamide adenine dinucleotide (NADH) dehydrogenase subunit 5	Yes. Janssen <i>et al.</i> , 2016	Yes	Rashidifard <i>et al.</i> , 2019c; Pretorius, 2018	Can reliably identify major lineages of tropical/thermophilic root-knot nematodes species. Better suited for use in sequencing than COI & COII genes	Needs to be combined with other molecular and morphological techniques to ensure accuracy
D2-D3	Yes. Palomares-Rius <i>et al.</i> , 2007; Subbotin <i>et al.</i> , 2006	Yes. Subbotin <i>et al.</i> , 2008; Al-Maafi <i>et al.</i> , 2003; Banna <i>et al.</i> , 1997	Rashidifard <i>et al.</i> , 2019c; Onkendi and Moleleki, 2013a	Requires a single individual for DNA extraction. Used to design and verify results from other techniques e.g., SCAR-PCR	Time consuming. Requires a well-curated database for true accuracy.

Genotyping by sequencing (GBS)	Yes. Vuong <i>et al.</i> , 2021; Castagnone-Sereno <i>et al.</i> , 2019; Elshire <i>et al.</i> , 2011	Yes. Poland and Rife, 2012; Thompson, 2008	Rashidifard <i>et al.</i> , 2018	Simple protocol. Reduces sequencing costs, while obtaining genome-wide information. No reference sequence limits	Very few limitations, but limited use of this method for <i>Meloidogyne</i> identification exists. Methods and quality of protocols and results are continuously improving
Spacer Regions: Internal Transcribed spacer (ITS). External transcribed spacer (ETS). Intergenic region spacer (IGS)	Yes. Adam <i>et al.</i> , 2007; Palomares-Rius <i>et al.</i> , 2007; Wishart <i>et al.</i> , 2002; Blok <i>et al.</i> , 1997	Yes. Waeyenberge <i>et al.</i> , 2000; Al-Banna <i>et al.</i> , 1997; Ibrahim <i>et al.</i> , 1994	Rashidifard <i>et al.</i> , 2019c; Rashidifard <i>et al.</i> , 2018; Onkendi and Moleleki, 2013a, b	Several protocols are available for nematode identification	<i>M. arenaria</i> , <i>M. incognita</i> & <i>M. javanica</i> have very little differentiation in their ITS regions. Time consuming. Multiple types of ITS within a species/individual limits accuracy



Figures 2.8a & b: (a) Oesophageal region of mature root-knot nematode female; (b) Schematic illustration of the perineal-pattern of a mature root-knot nematode female (Illustrations adapted from Rashidifard, 2019; Karssen and Moens, 2006).

2.3 Other selected biological aspects of plant-parasitic nematodes

The life-stage development during the respective life cycles of *Meloidogyne* and *Pratylenchus*, and their reproduction strategies are important biological aspects that contribute towards the importance of these genera being respectively rated as the No-1 and No-3 economic most important plant-parasitic nematode genera (Jones *et al.*, 2013). Therefore, a tabularized summary of such information for these two genera is provided in Table 1.8.

Table 1.8: The life-stage development during the respective life cycles of *Meloidogyne* and *Pratylenchus*, and their reproduction strategies.

<i>Meloidogyne</i> ; sessile, endoparasites	<i>Pratylenchus</i> : vermiform and migratory, endoparasites
Life cycle	
Life stage stages	
According to Jones <i>et al.</i> (2013); Karssen <i>et al.</i> (2013) J1 develops within an egg and hatch as an infective, second-stage juvenile (J2). During feeding, the J2 become swollen and sedentary, followed by molts to third- (J3) and fourth-stage juveniles (J4) which do not have stylets and do not feed. The J4 molts to either males or females. Sexual dimorphism occurs due to the latter life stages and females being swollen and globose-like, while J2 and males are vermiform. Females produce eggs which are deposited in a gelatinous matrix (Escobar <i>et al.</i> , 2015). The life cycle ranges between 20 days - 30 days at temperatures of 25 °C - 30 °C (Greco and Di Vito, 2009; Shurtleff and Averre, 2000).	The life cycle can range between 4 - 8 weeks, depending on the environmental conditions and suitable plant hosts. Like <i>Meloidogyne</i> , it includes an egg, four juvenile stages and mature males and females. They are migratory endoparasites that enter the root and move through the root system while feeding. All life stages are vermiform and infective (Jones <i>et al.</i> , 2013).
Feeding site	
The J2 penetrates the roots of a host plant and migrates through the intercellular space to enter the vascular cylinder where it induces the formation of feeding sites. During feeding, the J2 become swollen and sedentary and form multinucleated giant cells in vascular tissue; the J3 and J4 developing from sessile J2 develop into a mature female that produces egg masses protected in gelatin matrices (Asmus <i>et al.</i> , 2000). Root tip galls inflicted by <i>Meloidogyne</i> can be confused with galls produced by ectoparasites such as <i>Xiphinema</i> or with aluminium toxicity	Individuals migrate intracellularly through the root causing necrosis of root surface tissue and the cortex of plants (Davis and MacGuidwin, 2000). Lesions may overlap, can reach 1–2 mm in length and change colour to green and reddish-brown causing degradation of epidermal and cortical cells reducing the amount of root branching and hairs, and ultimately the plant's ability to access and translocate water and nutrients to aerial plant parts (Smiley, 2015; Vanstone <i>et al.</i> , 1998).

(Fourie, 2016, Potchefstroom, SA, personal communication).	
Reproduction strategies	
Two reproduction strategies exist, namely sexual (amphimixtic), and asexual (parthenogenetic) reproduction (Chitwood and Perry, 2009; Perry <i>et al.</i>, 2013). For amphimixis, the presence of males is obligatory, e.g., <i>Meloidogyne kikuyensis</i> De Grisse, 1961 (Karssen <i>et al.</i>, 2013), the female oocyte is fertilized by the male sperm and meiosis follows. Parthenogenetic occurs in the absence of males (e.g., <i>M. incognita</i>) where the eggs divide through meiosis for further development (Chitwood and Perry, 2009)	Reproduce through amphimixis where male and female individuals are present. However, in the absence of males, females can reproduce through parthenogenesis (Davis and MacGuidwin, 2000).

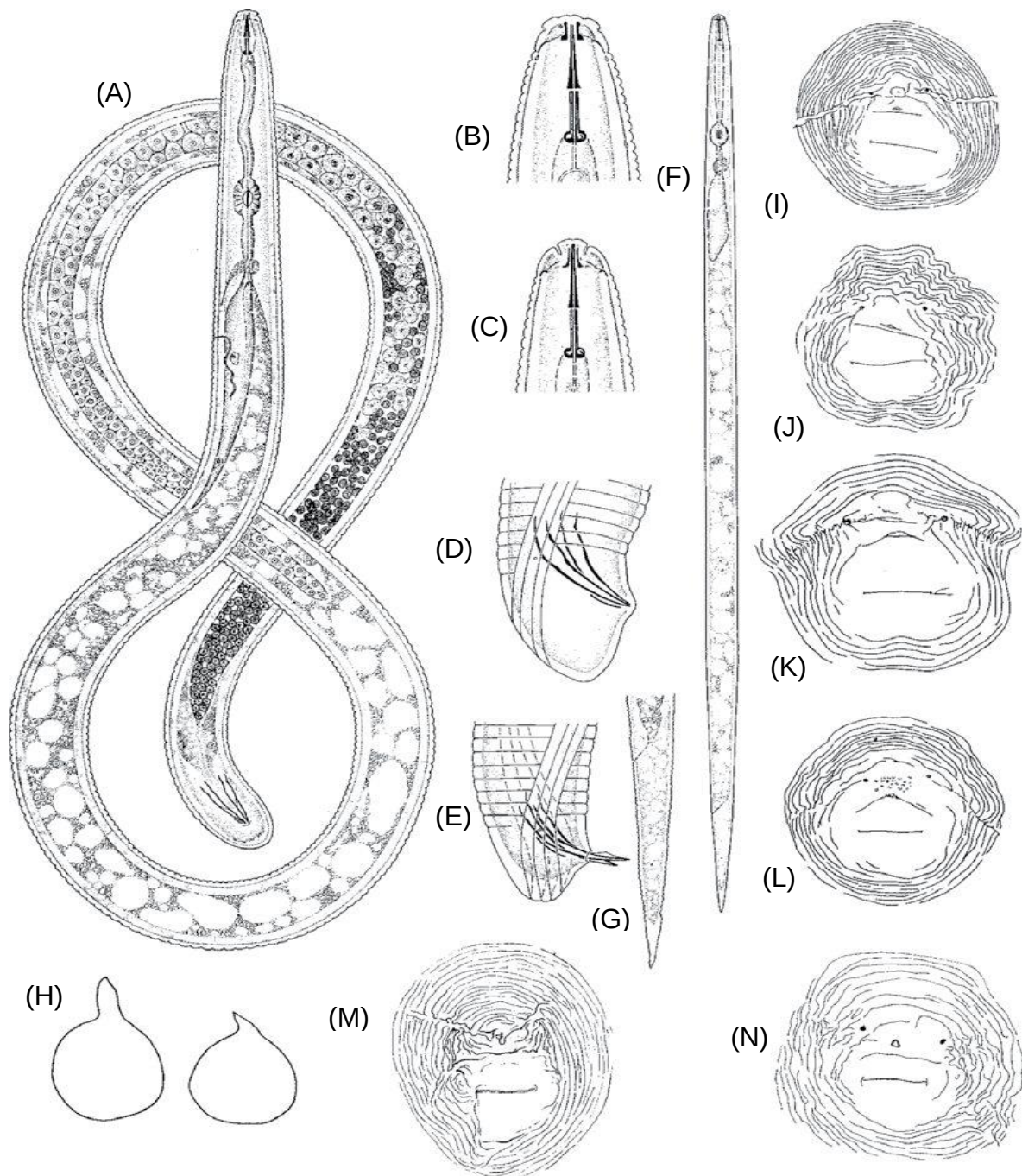


Figure 2.9: Line drawings of *Meloidogyne incognita*: (A) male; (B and C) male labial region; (D and E) male tail; (F) infective, second-stage juvenile (J2); (G) J2 tail; (H) mature females. Perineal-patterns of: (I) *Meloidogyne javanica*; (J) *Meloidogyne incognita*; (K) *Meloidogyne arenaria*; (L) *Meloidogyne hapla*; (M) *Meloidogyne graminicola*; (N) *Meloidogyne exigua* (drawings are for illustrative purposes only and are not to scale) (Hunt and Handoo, 2012)

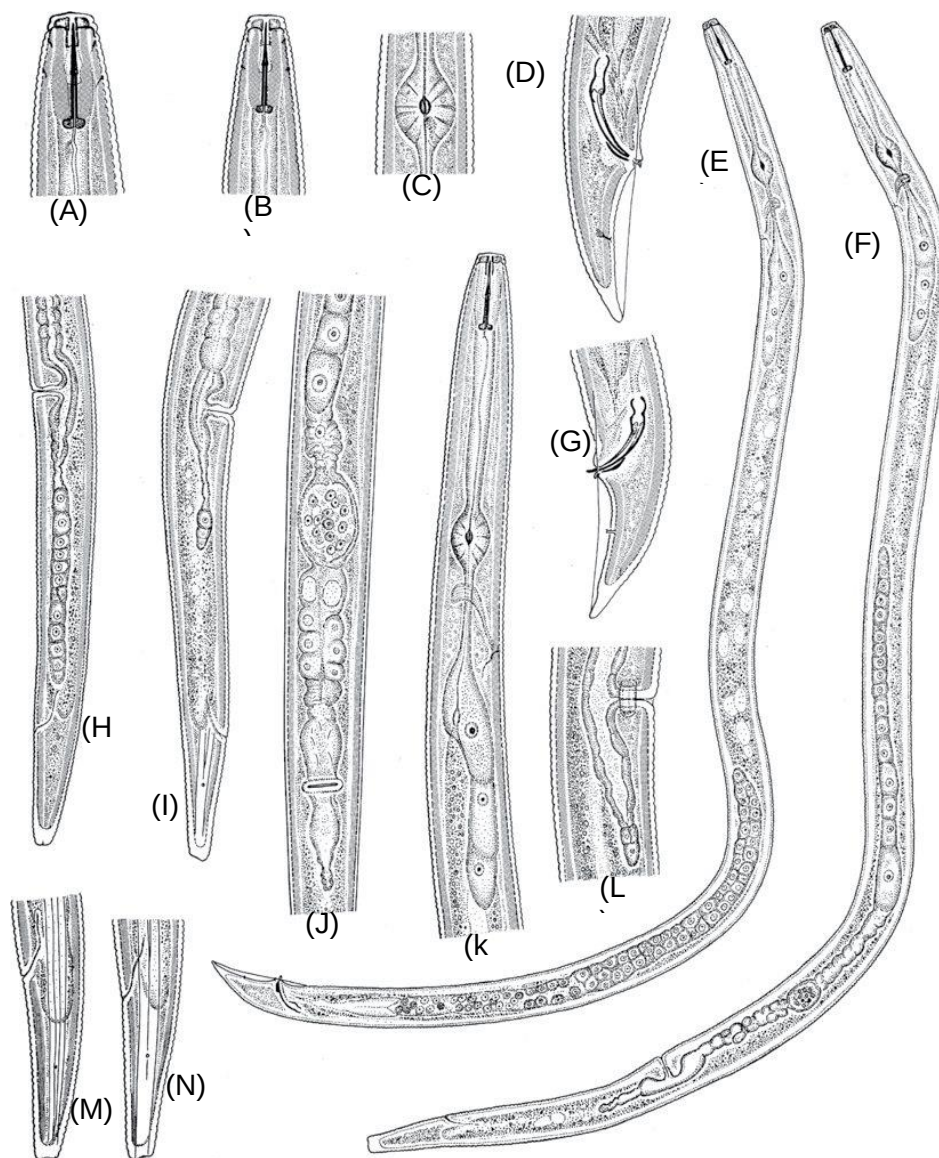


Figure 2.10: Line drawings of *Pratylenchus coffeae*: (A) female labial region; (B) male labial region; (C) median bulb; (D and G) male tail; (E) male; (F) female; (H and I) female posterior region; (J) female vulval region, ventral view; (K) pharyngeal region; (L) vulval region; (M and N) female tails (From Siddiqi, 1976, in CIH Descriptions Set, CAB International) (drawings are for illustrative purposes and are not to scale).

3.3 Management strategies

Worldwide strategies used to control plant-parasitic nematodes in grain crops include cultural practices, host plant resistance (induced or genetic) and crop rotation, chemical (Stapleton *et al.*, 2010) and biological control (Jones, 2017). Nematode management is aimed at reducing the number of nematodes present in the rhizospheres of crops or plants to below the economic threshold levels (Bridge and Starr, 2007). It is impossible to completely

eradicate nematode populations and thus, a small number of eggs and/or other life stages of plant-parasitic nematodes will survive irrespective of the strategies applied to control them (Engelbrecht, 2020; Lopes *et al.*, 2019; Onkendi *et al.*, 2014; Karssen *et al.*, 2013; Sirias, 2011; Moens *et al.*, 2009). General poor quality of agricultural soils (Hugo *et al.*, 2014) and ineffective use of biological control (Fourie *et al.*, 2016; Fourie *et al.*, 2014; Agbenin, 2011) promote plant-parasitic nematodes population. A summary of management strategies that help producers to keep nematode populations below economic threshold levels is discussed below.

3.3.1 Chemical control

Of late, many nematicides have been removed from the market because of their unacceptable toxicity profiles (DAFF, 2017; Haydock *et al.*, 2013) and their adverse impact on animals, humans, and the environment (Sikora *et al.*, 2005). Table 1.9 lists a range of chemical nematicides and seed treatments available for use on grain crops in South Africa.

Table 1.9: Represents the use of chemicals and seed treatments to reduce plant-parasitic nematode communities, mostly represented by root-knot nematodes and lesion nematodes in South African grain fields.

Grain crop	Trade name	Active substances
Maize	Curaterr 10GR®, Furadan®, Carbosan 100GR®, Carbofuran 100GR®, Carbodan®	Carbamate
	Carbosulfan	Carbamate
	Crop Guard®	Furfural (<i>aldehyde</i>)
	Counter®, Ortofos®, Terbufos, Terburops®, Terrafos®	Organophosphate
	AVICTA® 500FS	Abamectin
	Poncho®VOTiVo®	<i>Bacillus firmus</i>
	PI Plus	<i>Purpureocillium lilacinum</i>
	Biostart	<i>Bacillus</i> spp.
	VELUM® 1GR	Fluopyram
Soybean	VELUM® 1GR	Fluopyram

	AVICTA® 500FS	Abamectin
Sunflower	Curaterr®, Furadan®, Carbosan 100GR®, Carbofuran 100GR®, Carbodan®	Carbamate
	Counter®, Ortofos®, Terbufos, Terburops®, Terrafos®	Organophosphate
Wheat	Curaterr®, Furadan®, Carbosan 100GR®, Carbofuran 100GR®, Carbodan®	Carbamate

Walters (1979b) showed that the application of old-generation, synthetically derived nematicides substantially alleviated plant-parasitic nematode problems in maize crop production on sandy soils. The author also reported yield increases (ranging from 28% - 42 %) because of carbofuran application in the Free State Province where *P. zae* dominated. In the same study, 14 - 60 % increases in maize yields were recorded in plots that were fumigated with 1,3-dichloropropene (DD®). Application of aldicarb, however, did not result in significantly higher yields but suppressed plant-parasitic nematode population levels significantly (Mc Donald and De Waele, 1987b). Another study where application of products with active substances (a.s.) cloethocarb and carbofuran were applied, showed significant reduction of nematode pest complexes (comprising Dorylaimidae, *Meloidogyne* spp., *Paratrichodorus* spp., and *Pratylenchus* spp.) in two field trials in the Mpumalanga Province (Van Rensburg, 1988). Concurrently, maize yield increased because of cloethocarb applications ranging from 52% to 110 % compared to the untreated control, whereas for carbofuran it was 59 %. In 1996, the western maize production areas in SA conducted seven field experiments with granular nematicides over four seasons where *M. incognita* and *M. javanica* dominated as either single or mixed populations (Riekert, 1996). Yield increases, ranging from 50 to 500 kg ha⁻¹, were recorded in these experiments as a result of nematicide application. Currently, three new-generation, ‘softer’ products (minimal impact on natural resources, animals and humans) are registered on maize in SA, namely Avicta® 500FS (a.s. Abamectin) (<https://www.syngenta-us.com/seed-treatment/avicta-500-fs>), VELUM® 1GR (a.s. Fluopyram) (https://s3-us-west-1.amazonaws.com/agrian-cq-fs1-production/pdfs/Velum_Label.pdf) and CropGuard (a.s. Furfural (aldehyde)) (http://www.cropguard.co.za/downloads/Crop%20guard%20leaflet_English.pdf). The nematicide EDB® (AL; 1800 g⁻¹) was consistently found to reduce *M. javanica* population levels in two experiments, resulting in the lowest *M. javanica* population levels and highest yields (Fourie *et al.*, 2015; Fourie and Mc Donald, 2001).

The use of synthetic nematicides on soybean crops was not cost-effective as demonstrated by Fourie *et al.* (2015) and Engelbrecht *et al.* (2020) due to mainly the low yields experienced earlier. However, the use of aldicarb and terbufos treatments performed well while biological control agents (PI Plus; a.s. *Purpureocillium lilacinum* and Biostart; a.s. *Bacillus* spp.) were less effective in reducing *M. javanica* population levels. Four field experiments conducted where natural infestations of *M. incognita* occurred showed that significant differences ($P \leq 0.05$) existed among the 14 nematicide treatments evaluated population levels in roots (Fourie and Mc Donald, 2007). Only oxamyl SL, terbufos GR and Avicta® seed treatment resulted in a significant ($P \leq 0.05$) reduction of root-knot nematode population levels. These nematicides also generally resulted in a higher income per hectare compared to the control.

In 2021 VELUM® 1GR was registered on soybean in SA and it is currently the only product with nematicide activity available to producers. AVICTA® 500FS (a.s. abamectin) (<https://www.syngenta.com/protecting-crops/products-list>) is registered but logistical problems are experienced in treating the seed.

For sunflower, carbofuran and terbufos is registered as insecticides and for wheat, carbofuran is a registered nematicide locally (<https://www.agri-intel.com>). The value of using carbofuran on grain crops is that it is also an insecticide and has a dual action making it cost-effective for producers.

In areas where maize is produced, there is a common occurrence of irregular rainfall and poses a significant risk when using nematicide due to its high financial implications. Nematode damage in maize is hence often ignored until the infestation has become severe.

3.3.2 Genetic host plant resistance

Genetic host plant resistance describes the prevention and/or restriction/limitation of feeding, penetration, development, and reproduction of target plant-parasitic nematodes species because of gene expression (Fourie *et al.*, 2017). Walters (1979b) included different maize hybrids in experiments concurrent to the testing of nematicides and found variable genetic responses. All genotypes (e.g., cultivars, hybrids, and open-pollinated varieties) were identified as susceptible to a nematode pest complex that mainly consisted of *P. zaeae*. In a glasshouse experiment, Van Biljon and Meyer (2000) reported that maize cultivar SNK 2340 supported the highest population levels of *P. zaeae* and *P. delattrei* compared to tobacco (*Nicotiana tabacum*), weeping love grass (*Eragrostis curvula*), Rhode's grass (*Chloris gayana*), oat (*Avena sativa*), soybean, pearl millet (*Pennisetum glaucum*) and wheat. High

reproduction factor values obtained for *P. zeae* (ranging from 3 - 25) and *P. delattrei* (ranging from 30 - 143) demonstrated the high susceptibility of these maize cultivars.

Ngobeni *et al.* (2011) determined the host status of local commercially available maize genotypes for local, single-species populations of *M. incognita* race 2 and *M. javanica*, respectively. Hybrids DKC80-10 and AFG4410 were highly susceptible to both nematode species, while DKC78-15B, PHB3203 and DKC61-25B were resistant to at least one of the species. Also, an inbred line (MP712W) from the USA showed resistance (Windham and Williams, 1988; Aung *et al.*, 1990) to the SA species tested. This, however, poses a problem since *M. incognita* and *M. javanica* often occur in mixed populations in local maize-based production systems (Riekert 1996; Riekert and Henshaw, 1998). Also, genotypes resistant to one of these species, but susceptible to the other, can stimulate one species to dominate in a particular field and hence adversely affect successive crops (Ngobeni *et al.*, 2011). In a more recent study, 20 commercially available hybrids that were screened for their host status to *M. incognita* were all susceptible to the pest (Pretorius, 2018).

Soybean crops usually have none, low, moderate, or high levels of resistance to a target nematode species (Fourie *et al.*, 2017). The use of genetic resistance against plant-parasitic nematodes, such as root-knot nematodes and lesion nematodes, has been identified as one of the most cost-effective and environmentally friendly management strategies for these pests in soybean (Bridge and Starr, 2007). Fourie *et al.* (2001a) described substantial variation among 38 soybean cultivars in terms of their host status to *M. javanica* and *M. incognita* races 1, 2 and 4. The local cultivar Gazelle was identified with superior genetic resistance against *M. javanica*. Although these authors identified several cultivars with resistance to *M. javanica* and *M. incognita* races 1 and 4 using reproduction factor (Rf) as the main criterion (Windham and Williams, 1988), none exhibited resistance to *M. incognita* race 2. However, Van Biljon (2004) reported the poor host status of various commercially available soybean cultivars to several races of *M. incognita* and *M. javanica*. Fourie *et al.* (2006) identified genetic resistance to *M. incognita* race 2 in six local cultivars, with cultivar LS5995 being superior. Mbatyoti *et al.* (2018) performed similar work on soybean cultivars but only for *M. incognita*. Further research focused on the introgression of *M. incognita* and *M. javanica* resistance into conventional high-yielding commercial cultivars (Fourie *et al.*, 2015). Mbatyoti *et al.* (2018) verified that the superior *M. incognita* resistance exhibited by LS59950 and some of its progeny (into which the trait has been introgressed) was not compromised by an increase in the temperature regime of between 3 and 4 °C. However, the same study also showed that the *M. incognita* densities in the roots of susceptible cultivars increased under the increased temperature regimes to which soybean crops are likely to be exposed with climate change becoming a reality. Furthermore, such research is

dated since new cultivars enter the market on an annual basis. Additionally, introgression of resistance traits should be done for Roundup Ready® cultivars, as they dominate the local market (Mbatyoti *et al.*, 2020). In SA, more than 90% of soybean cultivation used glyphosate-tolerant cultivars during the 2017 growing season (Khojely *et al.*, 2018).

Limited and fragmented information exists for the host status of sunflower and wheat to root-knot nematodes and lesion nematode species in SA. According to Bolton and De Waele (1989) reported that four sunflower cultivars screened for their host status to *P. zeae* in both field and glasshouse experiments where natural infested with this nematode species, and all cultivars identified as non- or poor hosts compared to maize and grain sorghum genotypes identified as susceptible. Pretorius *et al.* (2014) reported that 20 commercially available cultivars were all susceptible to *M. incognita*. Steenkamp *et al.* (2015) showed resistance against one root-knot nematode species *M. incognita*. Substantial variation existed among the sunflower cultivars in terms of their host status to the two respective root-knot nematodes species, cultivar Procol 101CL only showed resistance to *M. incognita* since it had a mean Rf of 0.5, ELF-index value of 0.6 and 2 534 eggs and J2 per root system. None of the sunflower cultivars tested, however, exhibited resistance to *M. javanica*. This sunflower cultivar can be included in the sunflower breeding programme to breed for root-knot nematodes resistance. Ultimately, it can be used by producers to reduce population levels of *M. incognita* where it poses problems and this way contributes towards sustainable crop production (Steenkamp *et al.*, 2015).

Van Biljon and Meyer (2000) reported that in a glasshouse host suitability experiment, wheat cultivar SST 825 maintained low population levels of both *P. zeae* and *P. brachyurus* (reproduction factor (RF) values <1), indicating the poor host status of the cultivar. Moreover, Steenkamp *et al.* (2015) tested the wheat cultivars against root-knot nematodes and lesion nematodes, however, none exhibited resistance to the species.

Unfortunately, screening of cultivars to the main plant-parasitic nematodes species causing problems on grain crops grown in SA is lacking incentives to breeders of seed companies or even public breeding institutions (Mc Donald and Nicol, 2005). Moreover, until recently cultivars of mainly maize, soybean, and sunflower have been evaluated for their host status to only root-knot nematodes species, while assays with lesion nematodes species were not done (Fourie, H, personal communication, Potchefstroom, November, 2022). According to the latter author's personal communication, this situation is changing and hopefully, cultivars of grain crops will in the future be screened against both the mentioned plant-parasitic nematodes genera although it will most probably continue on a relatively small scale due to lack of funding. The desperate need to find grain crops genotypes that are resistant to multiple nematode species is becoming more urgent since genetic host plant resistance to

root-knot nematodes represents one of the few cost-effective and environmental-friendly management strategies for reducing yield losses caused by these pests in grain crops (Bridge and Starr, 2007).

3.3.3 Crop rotation and alternative hosts

In SA, maize monoculture was the common practice and large-scale expansion of the maize market followed due to the success of the green revolution (Borlaug *et al.*, 1969). However, the negative effects of crop rotation were emphasised by Riekert and Henshaw (1998) when rotating maize (cultivar PAN6043) with oilseed crops such as cowpea (*Vigna unguiculata* L.), groundnut (*Arachis hypogaea* L.) and soybean in sandy soil in the Free State province. Substantial increases (up to 189-fold) in population densities of a mixed *M. incognita* and *M. javanica* community (70:30 ratio) were experienced when cowpea cultivar Glenda and soybean cultivar Knap were included once in a maize-based rotation system over four consecutive growing seasons. A 3-fold increase in root-knot nematode population densities in maize-soybean rotations was recorded. Associated yield losses of 44 % after one groundnut rotation (cultivar Sellie), 55 % after one soybean rotation, and 60 % after one cowpea rotation demonstrated the adverse effect of the root-knot nematode community for such cropping sequences. In addition, several other crops that are commonly rotated with maize have been confirmed either to host especially root-knot nematode species that could damage maize or the other grain crops (soybean, sunflower, wheat) that are commonly rotated with it. However, Bolton and De Waele (1989) reported that maize could be rotated with sunflower to reduce *P. zae* populations. The authors also warned that when *M. incognita* and *M. javanica* are present in such fields, the rotation of maize and sunflower is not desirable.

Ntidi *et al.* (2015; 2012) demonstrated that weeds commonly found in maize fields are susceptible to root-knot nematodes and serve as susceptible hosts of these pests. Hence timely and effective weed control should form an integral part of good production practices. In addition, crop rotation is generally not an effective strategy to reduce root-knot nematodes or lesion nematodes populations in maize-based cropping systems due to their polyphagous nature (Nicol and Rivoal, 2008). The use of poor host or root-knot nematodes resistant cultivars of rotation crops should, however, be the main strategy to combat root-knot and lesion nematodes problems in maize-based production systems. Mc Donald and Nicol (2005) stated that triticale cultivars Abacus and Muir hosted fewer lesion nematodes than bread or durum wheats but that rotation crops had limited effectiveness against lesion nematodes because of their polyphagous nature (Nicol and Rivoal, 2008).

3.3.4 Alternative control options with focus on biological control

A purposeful drive towards investigating safer and less expensive management alternatives to mitigate the negative effect of predominant plant-parasitic nematode pests associated with local grain crops is experienced. Evaluation of alternative strategies with potential bionematicide action commenced in SA during 1980s with a seaweed concentrate (De Waele *et al.*, 1988) and various herbicides (Jordaan and De Waele, 1988). The seaweed product impaired the reproduction of *P. zeae* in an *in vitro* experiment by significantly reducing (by 47% - 63 %) but a phytotoxic effect was evident on maize plants, which supposedly made plants more susceptible to infection by *P. zeae*. Currently, only one biologically-based seed-coat product is registered on maize in SA, namely, Poncho®VOTiVo® (a.s *Bacillus firmus* (<https://www.agro.basf.co.za/en/Products/Overview/>)). For soybean, sunflower and wheat no biologically, based products are registered for use under SA environmental conditions (<https://www.agri-intel.com>).

The use of bacterial and fungal strains is an alternative management strategy for root-knot nematode and lesion nematode infections (Dias-Arieira *et al.*, 2018; Ashoub and Amara, 2010) and can economically reduce the damage done to important grain crops. Bacteria known to exhibit nematicidal activity against root-knot nematodes and lesion nematodes populations include several species of *Bacillus* as well as *Pseudomonas* and *Pasteuria*, including (*B. megaterium*, *B. cereus*, *B. nematocida*, *B. subtilis*, *B. thuringiensis*, *B. pumilus*, *B. firmus*, *B. amyloliquefaciens*, *Pseudomonas fluorescens*, and *Pasteuria thornei*) (Engelbrecht *et al.*, 2018; Watson *et al.*, 2018; Jamal *et al.*, 2017; Lee and Kim, 2016; Machado, *et al.*, 2016). In addition, *Bacillus* and *Pseudomonas* spp. used as biological control agents are safe for the environment and non-target organisms (animals, humans, and other soil-dwelling organisms) (Gao *et al.*, 2016). The ultimate approach should be that producers apply an integrated pest management (IPM) strategy to protect grain crops against plant-parasitic nematodes. This can be done by complementing the different strategies above so that it is still cost-effective.

3.4 Beneficial nematodes

Keetch and Buckley (1984) set the cornerstone in terms of listing plant-parasitic nematodes associated with the rhizospheres of crops and ornamentals in southern Africa. In 1987, nematologists of South Africa (SA) initiated the creation of the South African Plant Parasitic Nematode Survey (SAPPNS) representing a database that aimed to make a comprehensive assessment of the biodiversity of the local nematode fauna. This was done by compiling a catalogue of the plant-parasitic nematodes of SA (Marais, 2002). Adding significant and extensive information to the database was made by Kleynhans *et al.* (1996).

Concerning grain crops, various authors added valuable information about nematode-crop association to the SAPPNS. For example, for maize (*Zea mays* L.), Bekker *et al.* (2022), Pretorius (2018), Visagie *et al.* (2018) and De Waele and Jordaan (1988). Concerning soybean (*Glycine max* (L.) Merr), Engelbrecht *et al.* (2020), Mbatyoti *et al.* (2020; 2018) and Fourie *et al.* (2001a) contributed by adding nematode-survey data. Limited and dated information about nematode-crop associations, however, exists for sunflower (*Helianthus annuus* L.) (Bolton *et al.*, 1989) and wheat (*Triticum aestivum* L.) (Jordaan *et al.*, 1992).

Beneficial nematodes have also, but to a lesser extent than plant-parasitic nematodes, been studied as an objective of this survey project since scanty information about the general state of grain producers' fields in SA exists in this regard. The only studies that have been done represent those for maize (Bekker *et al.*, 2022) and soybean (Girgan *et al.*, 2020b; Mbatyoti *et al.*, 2018). This project hence provided a valuable opportunity to study the use of beneficial nematodes in rhizospheres of grain crops by using these organisms as biological indicators. Moreover, beneficial nematodes play important roles in ecosystem processes and functions also sensitive to environmental changes caused by anthropogenic disturbances and recovery (Zhong *et al.*, 2017; Sánchez-Moreno and Ferris, 2018; Fischer *et al.*, 2016; Rossouw *et al.*, 2008; Neher, 2001). Agricultural intensification and extensive use of pesticides and nematicides (Ferris *et al.*, 2012; Sánchez-Moreno *et al.*, 2006) can negatively affect the diversity and abundance of nematodes (García-del-Pino and Morton, 2010; Dewar *et al.*, 2000) and microbial communities (Jacobsen and Hjelmsø, 2014).

Terrestrial, beneficial nematodes present in agricultural crop fields are represented mainly by bacterivores, fungivores, omnivores, and predators (Colombo *et al.*, 2014; Kleynhans *et al.*, 1996; Yeates *et al.*, 1993). Nematode taxa are further classified into five categories along a r-k strategists scale (based on reproduction rates) and a colonizer-persister (c-p) scale ranging from c-p 1 (extreme r-strategists) to c-p 5 (extreme K-strategists) (Ferris and Bongers 2009; Ferris *et al.*, 2001). The information generated about the r- and K-strategists and based on c-p values are useful in interpreting the status of the soil food web in different habitats. It is also a powerful tool to assess the structure, function, and resilience of a soil food web (Du Preez *et al.*, 2022).

The following nematode-based indices (NBIs) are often used to classify the health of soils using nematodes as bioindicators, *viz.* The maturity index (MI), enrichment index (EI), Structure index (SI), basal index (BI), and channel index (CI) (Du Preez *et al.*, 2022; Ferris *et al.*, 2001). Furthermore, nematode metabolic footprints measure the magnitude of ecosystem functions and services (*i.e.*, facilitating carbon and energy flow in soil food webs) provided by nematodes (Ferris, 2010). Data generated for beneficial nematodes can be analysed using the Nematode Indicator Joint Analysis (NINJA) web-based tool (available at

<http://spark.rstudio.com/bsierieb/ninja>) (Sieriebriennikov *et al.*, 2014). This analysis is based on an estimator of a nematode's contribution to the different processes in an ecosystem or food chain and contains production and respiration components. It can also be used as an index of the carbon utilisation of nematode taxa and is a valuable expansion and integration of the soil food web analysis (Ferris, 2010; Ferris *et al.*, 2001) as illustrated in Fig. 2.11.

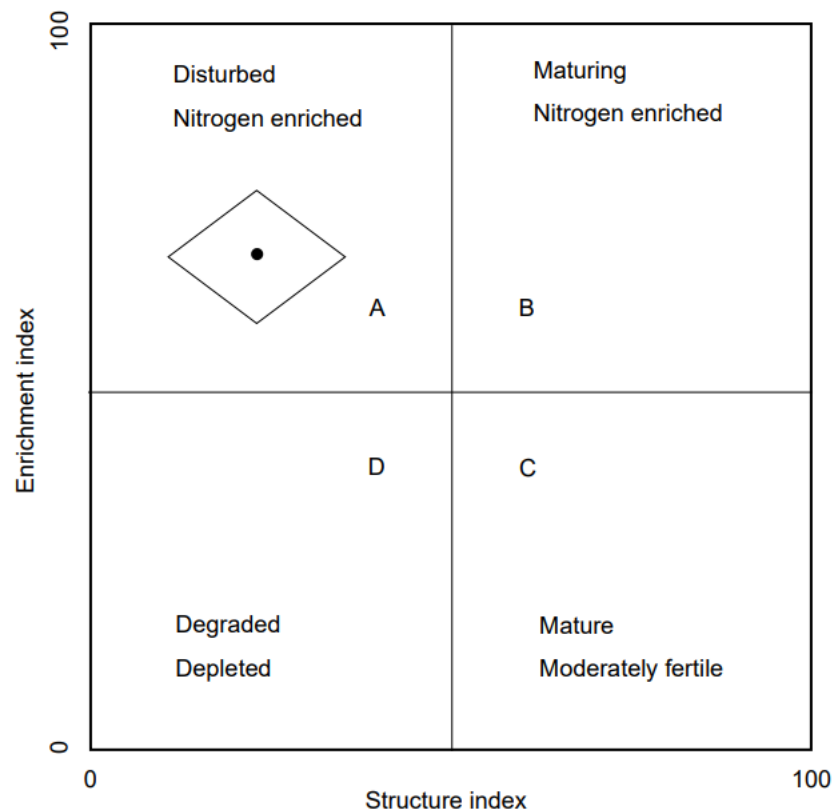


Figure 2.11: A schematic representation of the nematode faunal analysis (Illustration: Ané Loggenberg, NWU, 2021).

Chapters 1 and 2 of the dissertation serve as the foundation for the rest of this MSc study. These chapters provide background information on various aspects of the topic being explored. The conclusion drawn from this section is that the issue of nematodes in South African grain fields is a genuine concern. This study addresses the update needed on the predominant nematode pests and the current status of the soils concerning beneficial nematodes.

CHAPTER 2: METHODOLOGY

2.1 Introduction

This chapter gives a detailed overview of the methodology used to conduct a nematode survey in 18 grain-based farmlands in the Free State, Gauteng, and North West provinces of South Africa. The focus of this chapter is on techniques applied to conduct meaningful nematode sampling (root and soil samples), extraction, counting, and identification (classical/morphological and molecular approaches used) of the economically important nematode pest genera prevailing in such farmlands. In addition, information on the abundance and identify of the beneficial, non-damaging/non-parasitic nematodes in soils of the farmlands sampled were also obtained and used as a bio-indicator of soil health.

2.2 Survey

Roots and rhizosphere soil samples of grain crops were sampled from 18 fields of grain producers situated in local maize-based production areas in three provinces, viz. Free State, Gauteng and North West during the 2020 and 2021 growing seasons (Fig. 2.1a) as listed in Table 3.1. Sampling was done approximately 110 days after planting of the crops. For each of the fields sampled, eight representative root and corresponding soil samples were obtained in six areas (covering 50m x 50 m) per field using a zig-zag sampling method (Fig. 2.1b; Marais *et al.*, 2017; Kleynhans *et al.*, 1996). The eight samples obtained for each area were combined to make one composite sample, with six composite samples ultimately obtained per field.

A garden spade was used to remove the root systems of the crop plants along with the soil surrounding the roots and put into labelled plastic bags. The samples were preserved in cool bags and kept out of direct sunlight, transported to the Nematology Laboratory of the North-West University (Potchefstroom) and stored in a cold room (6 °C – 10 °C) until nematode extractions continued. This procedure was done usually within 5 days after the samples were collected. Rainfall and temperature data were obtained from the producers in whose fields the samples were taken and from the website Climate-data.org (2017). Cultivars planted by producers are listed in Table 2.1, but info in this regard was not supplied by all producers.

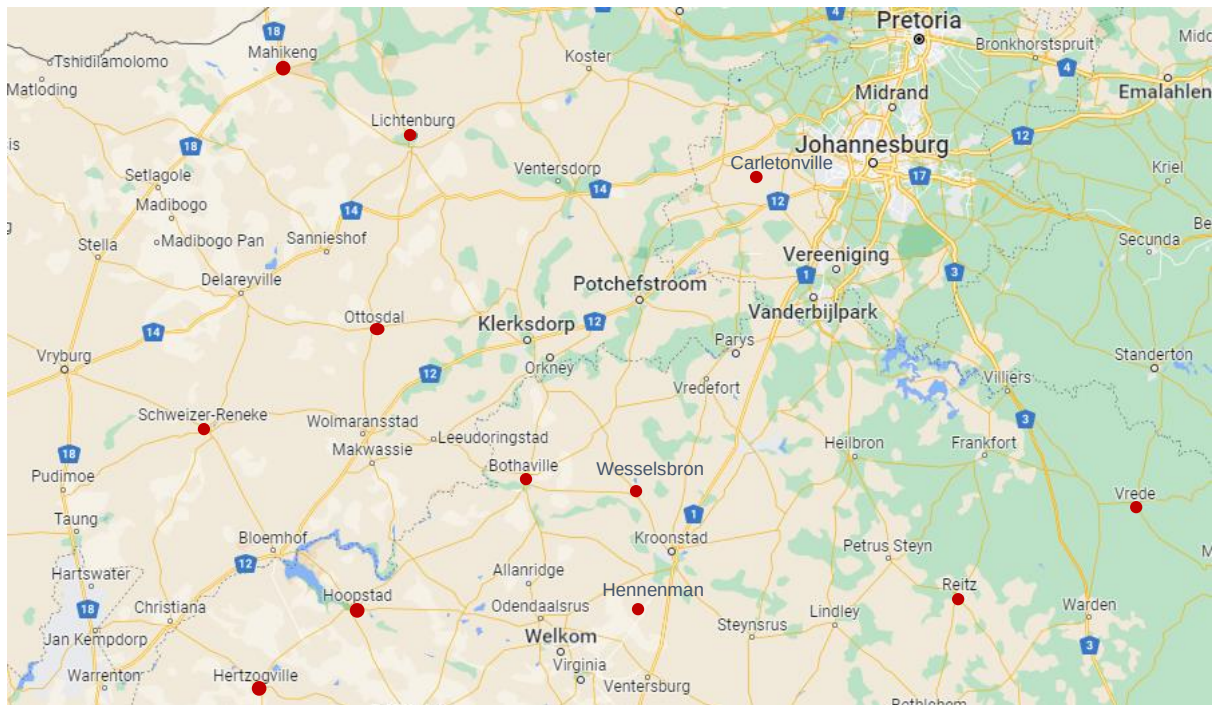


Figure 2.1a: A map of the maize production areas in the Free State, North West and Gauteng provinces showing where the fields (indicated by a red dot) sampled for nematode analyses during the 2020 and 2021 growing seasons were located. One dot may represent more than one field sampled in that area (<https://www.google.com/maps/@-27.0004877,26.44631,8.35z>).

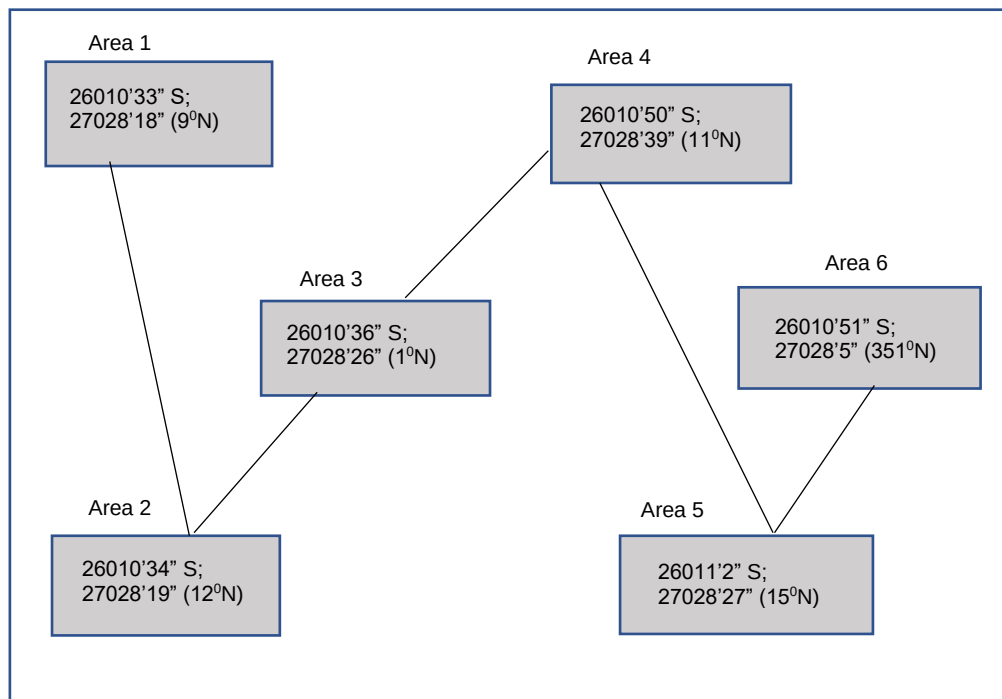


Figure 2.1b: An illustration of the six areas per field where samples were taken in a zig-zag pattern from (SH, Carletonville) farmlands of a grain producer for nematode analysis in Gauteng provinces during the 2020 and 2021 growing seasons (Illustration: Bongiwe Mhlongo, NWU).

Table 2.1: The information about 18 fields where nematode samples of grain crops were obtained during the 2020 and 2021 growing seasons.

FIELD NO & CODE	AREA	PROVINCE	CROP GROWN IN 2020	CROP GROWN IN 2021	%CLAY	RAINFALL 2020	RAINFALL 2021	NEMATICIDE USED	DRYLAND IRRIGATION /
1SH	Carletonville	Gauteng	Wheat	Soybean	25-35%	600	580	No nematicide	Irrigation
2DM	Lichtenburg	North-West	Sunflower	Maize	18-20%	736	678	Avicta® 500FS	Dry land
4CS_1	Hoopstad	Free State	Maize_Field 1	Maize_Field 1	<5%	400	430	Velum® 1G	Dry land
4CS_2	Hoopstad	Free State	Maize_Field 2	Maize_Field 2	<5%	400	430	No nematicide	Dryland
5JM	Bothaville	Free State	Maize	Maize	5%	600	750	Counter & Velum® 1G	Dry land
7WM	Henneman	Free State	Sunflower	Mielies	±12%	530	605	No nematicide	Dry land
8JB	Hoopstad	Free State	Wheat_Field 2	Soybean_Field 2	<8%	550	590	Velum® 1G	Dry land
10MG	Henneman	Free State	Maize	Maize	10%	541	457	Avicta® 500FS	Dry land
11IM	Hoopstad	Free State	Sunflower	Sunflower	5-10%	380	530	No nematicide	Dryland
13PVZ	Bothaville	Free State	Maize	Maize	0-5%	520	500	Terbufos and Avicta® 500FS	Dry land
14DV	Bothaville	Free State	Maize	Maize	8%	753	371	Avicta® 500FS	Dry land
16SJ	Bothaville	Free State	Maize	Maize	0.5%	800	374	Avicta® 500FS	Dry land
17JB_1	Bothaville	Free State	Maize	Soybean	12-15%	681	250	No nematicide	Dry land
17JB_2	Bothaville	Free State	Soybean	Maize	12-15%	681	250	No nematicide	Dry land
17JB_3	Bothaville	Free State	Wheat	Maize	12-15%	681	250	No nematicide	Dry land
18JF	Bothaville	Free State	Maize	Maize	12%	590	450	Curaterr & Velum® 500FS	Dry land
20NR	Ottosdal	North-West	Sunflower_Field 1	Maize_Field 1	16%	640	585	Avicta® 500FS & carbofuran	Dry land
22PZ	Vrede	Free State	Maize	Soybean	10-30%	270	300	No nematicide	Dry land

2.2.1 Nematode extractions and counts for field samples

2.2.1.1 The Riekert's adapted NaOCl method for extractions of eggs and second-stage juveniles (J2) of root-knot nematodes (root-knot nematodes) and endoparasitic nematodes from (5 g and 50 g) roots samples

Root-knot nematode eggs and J2 were extracted from 50 g root samples of the four crops sampled maize (*Zea mays* L.), soybean (*Glycine max* L.), sunflower (*Helianthus annuus* L.) and wheat (*Triticum aestivum* L.) using an adapted 1% sodium hypochloride (NaOCl) method (Riekert, 1995). Other endoparasitic nematodes were extracted from 5 g root subsamples using the sugar centrifugal-flotation method (Marais *et al.*, 2017).

The adapted NaOCl extraction method is specifically developed to extract the eggs of sessile endoparasitic nematode genera such as *Meloidogyne* (Riekert, 1995). The method is based on the use of NaOCl which acts as a dissolving chemical for the gelatinous matrix in which root-knot nematode (root-knot nematodes) females deposit their eggs in egg masses. The procedure followed during the extraction of root-knot nematode eggs and J2 was as follows and is illustrated by detailed infographics (Fig. 2.2):

- a) A 50 g representative sample was obtained from each composite root sample. It was first washed to remove excess debris and then cut into 0.5 -1 cm pieces and mixed thoroughly.
- b) The root samples were shaken for 4 min in 400 ml of a 1% NaOCl solution.
- c) The NaOCl mixture, containing mostly eggs, but also J2, was decanted on a 250 µm sieve that was nested on a stacked range of sieves, which from top to bottom consisted of 75-, 63- and 20 µm sieves.
- d) Root fragments on the top 250 µm sieve were washed thoroughly for about 4 min with running tap water.

Eggs and J2 were collected on the 20 µm sieve and washed (with approximately 10 ml of tap water) into a 100-ml sample bottle for counting.

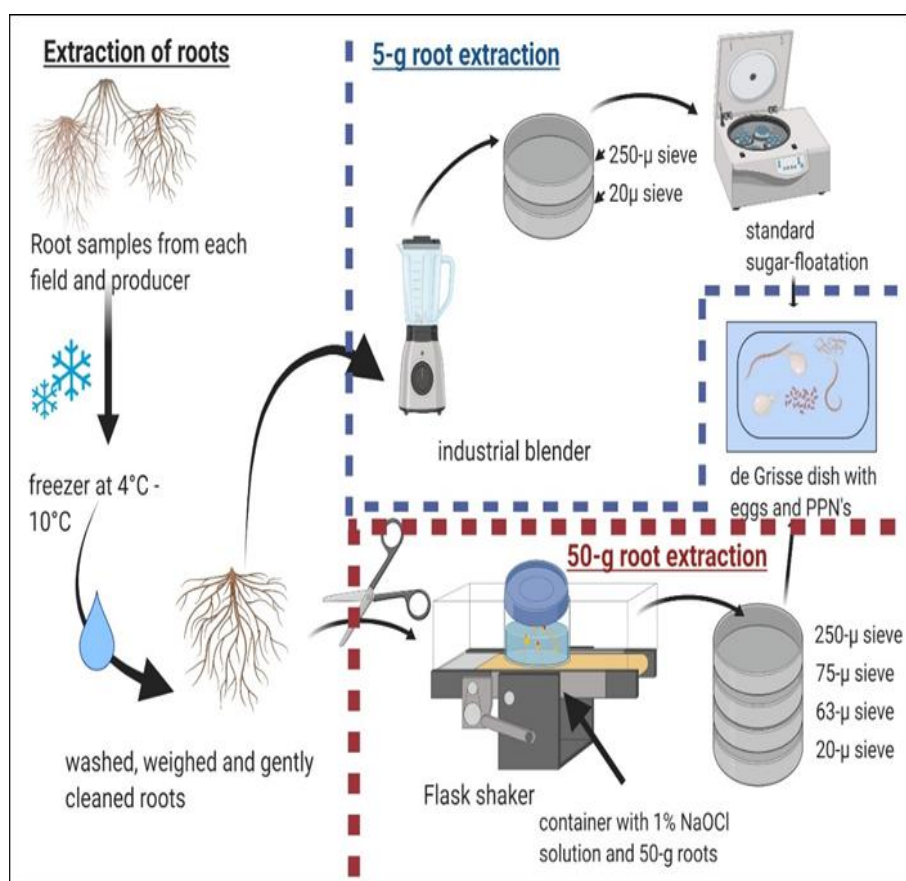


Figure 2.2: An infographic demonstrating the extraction of nematodes from roots (5 g and 50 g) (Created by Raymond L. Collett, NWU, Potchefstroom) (Marais *et al.*, 2017).

2.2.1.2 The adapted centrifugal-flotation method for the extraction of a wide range of plant-parasitic nematodes from plant roots (5 g) samples

This method is based on the gravity of nematodes and was followed according to the centrifugal-flotation method for plant material (Kleynhans *et al.*, 1996; Marais *et al.*, 2017). Concurrently, when obtaining 50 g roots for the NaOCl explained above (section 2.2.1.1), a 5 g root sample was obtained, cut into approximately 0.5-1-cm pieces and added to 250 ml tap water after which it was shredded in a domestic kitchen blender at a high speed for 90 s. The root suspension was then washed through a 250 μm sieve that was placed on top of a 25 μm sieve, using a gentle stream of running tap water. The residue left on the 25 μm sieve was collected and decanted into a 50 ml centrifuge tube. Next, approximately 2 cm³ kaolin was added to the water suspension containing the nematodes. Kaolin is a clay mineral that forms a layer and seals off the nematodes at the bottom of the centrifuge tube (Coolen and D'Herde, 1972). The content in the centrifuge tube was then centrifuged at 1 800 rpm for 3 min and the supernatant was decanted and disposed of. The centrifuge tube, with the nematodes sealed off at the bottom, was next filled with a sucrose solution (624 g sugar / 1000 ml water), the content was stirred well to break the kaolin layer and enable the nematodes to get into suspension. The tube was then centrifuged again at 1 800 rpm for 1 min

only. This was done to avoid exposing the nematodes to the sucrose solution for too long because they could become distorted and/or degraded. The supernatant was then decanted on a 20 µm sieve and rinsed thoroughly using a gentle stream of tap water. The nematodes were washed from the 20 µm sieve into a 100 ml plastic container for counting and identification.

2.2.1.3 The decanting and sieving method with sugar centrifugal-flotation method for extraction of nematodes from (200 g) soil samples

Nematodes were extracted from soil subsamples (200 g) by using the decanting and sieving methods, followed by an adapted sugar centrifugal flotation method (Marais *et al.*, 2017). Each of the six sub-samples of soil obtained per field was weighed to obtain 200 g, which was transferred to a plastic container (750 ml capacity) and soaked in 500 ml tap water for 60 min. The soil solution was then poured into a 2-litre jug and filled to 1 000 ml using pressured tap water to bring the nematodes into suspension. After letting the suspension stand for 15 s, to allow heavy soil particles to settle, the water with the suspended nematodes' fine particles were decanted through a coarse-meshed 2-mm sieve stacked on top of a 5-litre capacity, plastic container. This process was repeated three times (representing the adapted part referred to above) to ensure that nematode isolation from each soil sample was optimal. The content on the 2-mm sieve was rinsed thoroughly with tap water and the contents on the sieve discarded and the sieve properly cleaned by washing it with tap water. The nematodes and fine particles that were now present in the 5-litre container was slowly transferred to a 25 µm sieve and then decanted into a 200 ml glass beaker. The contents of the glass beaker were thoroughly mixed and evenly transferred to four 50 ml capacity centrifuge tubes to ensure that the centrifuge was balanced (the soil: water ratio of the tubes did not exceed a ratio of 3:1). One teaspoon (5 g) of kaolin was added to the content of each centrifuge tube and mixed thoroughly with a spatula, then centrifuged for 5 min at 3500 rpm. Kaolin helps the formation of a pellet at the bottom of the tube containing larger unwanted particles, while the lighter organic matter remains in suspension. After centrifugation, the supernatant was decanted from the centrifuge tube, and a sugar-water solution (1.15 g/cm³) was prepared by dissolving 1.6 kg of sugar in 2 L water. Then the sugar solution was added to the centrifugal tubes and mixed thoroughly. The content of the centrifuge tube containing the nematodes was then centrifuged for only 1 min at 3500 rpm, because the nematodes could become distorted if left too long in the sugar solution (Marais *et al.*, 2017). The sugar-water solution in which the nematodes were suspended was next decanted onto 25 µm sieve and the nematodes on the sieve rinsed well with tap water after which they were collected in a 100 ml sample bottle (with a final volume of 20 ml) for counting and identification (Fig. 2.3).

The extracted nematode suspensions obtained from the 5 g, 50 g roots and 200 g soil, were counted using a De Grisse counting dish (De Grisse, 1963) and a stereo microscope (Nikon SMZ1500) (60X magnification). Plant-parasitic nematodes, except for Meloidogyne, were identified to species level by the taxonomists of the Agricultural Research Council – Plant and Health

Protection (ARC-PHP, Pretoria, South Africa) while beneficial nematodes were identified to genus or family level using the protocols for nematode identification by Andr ssy (2009), Andr ssy and Gibson (2007), Andr ssy (2005) and Heyns (1971).

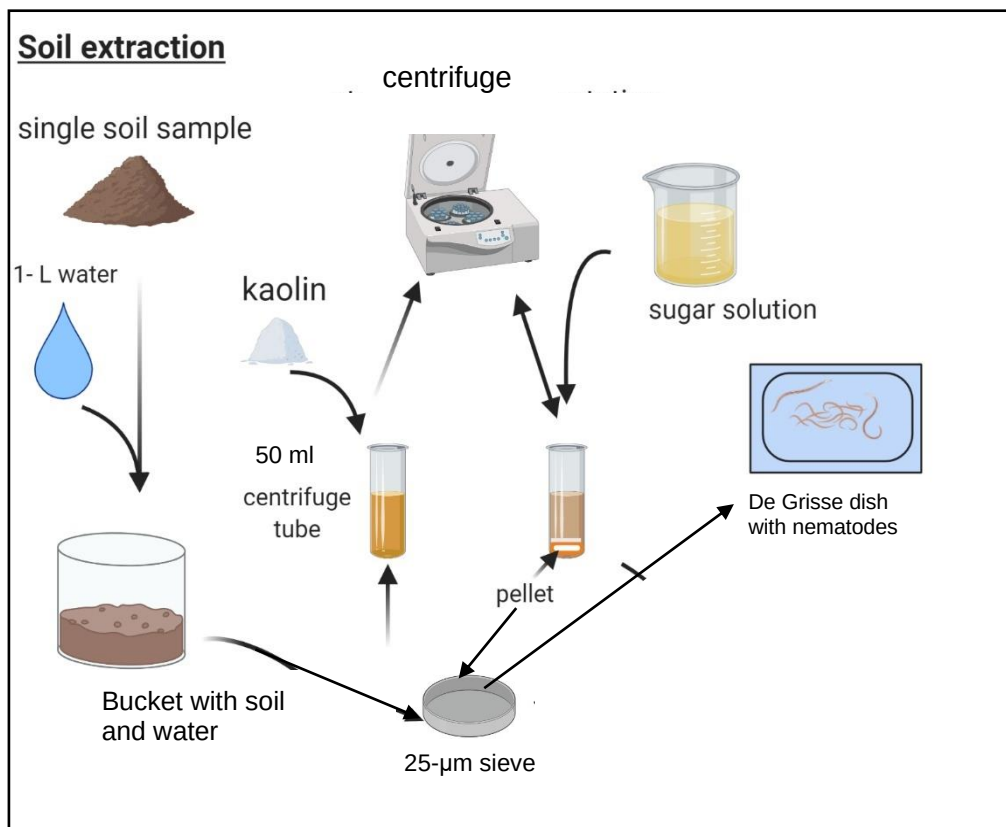


Figure 2.3: An infographic demonstrating the extraction of nematodes from soil samples (200 g) (Created by Raymond L. Collett, NWU, Potchefstroom) (Marais *et al.*, 2017).

2.2.2 Nematode fixation and mounting of slides

After counting the nematodes, individuals were isolated from the counting dishes using a tailor-made needle and fixed for mounting on permanent glass slides (50 X 50 mm) using the FPG fixative method. The latter solution contained 100 ml formalin 40%, 10 ml propionic acid, 890 ml distilled water and a pinch of picric acid (staining the nematode individuals yellowish). The fixation and mounting procedures are described stepwise below:

- The nematodes of each field were placed in separate syracuse dishes and most of the water covering them was drawn off. Then (80 ml) boiled tap water was added to a (100 ml) glass beaker and placed in the fume-hood and (depending on the number of nematode samples fixed). About (15-20 ml) FPG latter solution was drawn into a test tube using a pipette then placed into the boiled water until heated up to 60-70  C.

b) Then droplets of hot FPG fixative solution were poured onto the Syracuse dish with nematode individuals using a micropipette. The Syracuse dish was placed in a petri dish and closed with another petri dish (to prevent evaporation), then placed into a desiccator filled with FPG solution at the bottom. The desiccator was placed in an incubator for a minimum of 3 days at 38-40 °C.

After suspending the nematodes in the FPG fixative, the hydration step followed for which solution 1 and solution 2 were used. Solution 1 contained 200 ml 95% alcohol, 10 ml glycerin and 790 ml distilled water and Solution 2 contained 950 ml 95% alcohol and 50 ml glycerin. The hydration method was then followed as described stepwise below:

a) After taking out of the desiccator from the incubator, the syracuse dish was removed and half of the FPG fixative drawn off with a micropipette; then solution 1 was added to the syracuse dish containing the nematodes. The dish containing the nematodes was returned to the desiccator, in which a 95% alcohol solution was added to the bottom and left for 12 h.

b) The desiccator was removed from the incubator and the syracuse dish was removed from the desiccator. Then half of solution 1 was withdrawn as explained above and solution 2 added to the syracuse dish containing the nematodes; the dish was partially covered with a petri dish (to allow slow evaporation of the alcohol) and returned to the incubator (not in the desiccator) for 2-3 days or until all the alcohol was evaporated. After that, the Syracuse dish was placed for one day in a desiccator, which contained silica at the bottom, and then placed in the incubator. After this procedure was done, the fixed nematodes were fished from the Syracuse dish using a tailor-made needle and placed in tiny drop of glycerin on a microscope slide. The nematodes suspended in the glycerin were covered with a glass coverslip and the edges of the coverslip sealed with transparent nail polish (Cutex) to prevent drying out of the nematodes and/or leakage.

2.2.3 Identification of *Meloidogyne* spp.

2.2.3.1 Morphology

2.2.3.1.1 *In-vivo* rearing of *Meloidogyne* spp.

The eggs and J2 extracted from the root samples (see Paragraph 2.2.1.1) of the fields were each inoculated on roots of the root-knot nematodes susceptible tomato (cv Rodade) (Fourie *et al.*, 2012) and the *Meloidogyne* spp. populations (comprising single species) and/or communities (comprising more than one species) reared *in vivo* in a greenhouse. The tomato seedlings used were planted in individual 5-L capacity pots that were filled with fumigated sandy-loam soil (5.3% clay, 93.6% sand, 1.1% silt, 0.47% organic matter and pH (H₂O) of 7.47) four weeks before nematode inoculation. An ambient temperature regime (min. 19 °C – 21 °C and max. 25 °C – 27 °C) and a photoperiod of 14L: 10D were maintained in the greenhouse. Plants were carefully watered manually (to prevent cross contamination of *Meloidogyne* spp.) three times a week with

tap water using a 250 ml beaker. Infected tomato plants with visible galling were removed 56 days after inoculation (DAI) for morphological identification of the species present.

2.2.3.1.2 Staining of *Meloidogyne* spp. infected tomato roots for morphological identification using pharyngeal and perineal-patterns characteristics

After 56 days of in vivo rearing of the root-knot nematodes species obtained from the fields sampled, the infected tomato (cv Rodade) roots were harvested from pots in which each field was reared. The infected individual root systems containing galls were rinsed for 1 min under running tap water to remove soil and debris, and stained for 6 min using a sodium (Byrd *et al.*, 1983) by adding 100 ml NaOCl to 500 ml tap water (ratio = 1:5 NaOCl:water) into a glass beaker. The stained roots were removed from the latter solution and placed into 400 ml tap water for 15 min, after which it was rinsed with running tap water for 1 min. The rinsed roots were then transferred to a fume-hood where it was submerged for 30 s into a boiling solution consisting of 40 ml water and 1-ml acid-fuchsin solution (3.5 g acid-fuchsin dissolved in 250 ml acetic acid). Then, the root systems were left in this solution to cool down to room temperature for 30 min after which it was washed for 1 min under running tap-water and placed into a glass beaker containing 10 ml glycerol. After staining, the roots were inspected for mature females using a stereomicroscope (40× magnification) and such individuals were removed from the galled roots for morphological identification using perineal-pattern morphology and oesophageal characteristics (see Table 1.7) (Rashidifard *et al.*, 2019b).

2.2.3.2 Molecular identification

Rearing of *Meloidogyne* spp. was done using the same in vivo protocol as described in to rear the *Meloidogyne* spp. for morphological identification.

2.2.3.2.1 Deoxyribonucleic acid (DNA) extraction

Concurrently removing females for morphological identification, 20 mature females were removed from the root systems of each field for molecular identification. Individual mature females were gently removed from each root system with a sharp-point needle. For each field, 20 females were carefully transferred to a sterilised individual 1.5 ml Eppendorf by dislodging it in the tube near the bottom and then storing it in a fridge at 4 °C until DNA extractions were done. The DNA of root-knot nematodes females in each tube was extracted using the adapted procedure by Rashidifard (2019) from Musapa *et al.* (2013). This entailed that 20 µl chelex (5% w/v) and 3 µl proteinase K (20 mg.ml⁻¹) were added to each sterilised Eppendorf-tube, containing the root-knot nematodes mature females and vortexed for 10 s to homogenate the female tissue. The tubes were centrifuged for 30 s at 11000 rpm and incubated for 2 h at 56 °C followed by 10 min incubation at

95 °C. After this, the tubes were centrifuged for 30 seconds at 11000 rpm again to enable the release and purification of the DNA extracts of the females that were transferred to the tubes. The tubes with the female DNA were stored at -20 °C until used for the sequence characterised amplified region– polymerase chain reaction (SCAR-PCR) (Zijlstra *et al.*, 2000).

2.2.3.2.2 Amplification of deoxyribonucleic acid and gel electrophoresis

The procedure that was used for the sequence characterised amplified region–polymerase chain reaction (SCAR-PCR) represented that as published by Rashidifard (2019), Rashidifard *et al.* (2019c), Musapa *et al.* (2013) and Fourie *et al.*, (2001b). The DNA obtained for each *Meloidogyne* sp. was visualized using gel electrophoresis and conducted as described below.

Amplification of the DNA of the root-knot nematodes females was done using an Alpha Cycler 1 PCR Max thermocycler (Vacutec, Sheridan, Wyoming, USA). For each of the PCR reactions, a total volume of 25 µl was prepared and constituted of 18,5 µl (mixture of master mix (Promega Corporation, USA) and ddH₂O), 4,5 µl of DNA extracted from females of each of the fields sampled as well as 1 µl forward primer (10 µM) and 1 µl reverse primer (10 µM) of the respective root-knot nematodes species tested for. The species-specific primers for *M. arenaria*, *M. enterolobii*, *M. incognita* and *M. javanica* (Table 2.2a) were applied to identify whether these species were present or absent in the samples. The particular PCR conditions required for DNA amplification were programmed in the PCR machine to represent 2 min at 94 °C followed by 35 cycles of 30 s at 61 °C, 30 s (at annealing temperature and 1 min at 72 °C). Annealing temperatures were as follows for the respective species-specific primers: 61 °C for the Far/Rar, 64 °C for Fme /Rme and Fjav/Rjav primers, and 64 °C for Finc/Rinc primers (Long *et al.*, 2006; Zijlstra *et al.*, 2000) (Table 2.2b). The duration of this annealing process was 30 s for each of the set of primers.

After the process was finalized, the PCR products from root-knot nematode species obtained from each field sampled were loaded on a 2% agarose gel to determine whether root-knot nematodes species were present. Before loading the PCR products on the agarose gel, a 1 kb ladder (for *M. arenaria*, *M. enterolobii* and *M. javanica*) and a 100 bp ladder (for *M. incognita*) were loaded into the first well. This served as a baseline that indicated the band positions of the DNA fragments amplified for the root-knot nematodes species for each of the fields. From the 2nd well, 5 µl containing 2 µl of a GelRed loading dye (www.biotium.com) and 3 µl of the PCR product from the females of the respective fields were loaded after the solution was mixed thoroughly. The last two wells contained an untreated control (without root-knot nematodes template) and the PCR product of one of the four known root-knot nematodes species to which the DNA bands of the root-knot nematodes species from the fields could be compared. The gel was run for 45 min at 80 V and then the DNA bands on the gel was visualized and photographed using a UV transilluminator.

Table 2.2a: Identification of South African populations of *Meloidogyne arenaria*, *M. enterolobii*, *M. incognita* and *M. javanica* using the sequence characterised amplified region – polymerase chain reaction (SCAR-PCR).

Target spp.	Primer code	Primer sequence (5' →3')	Fragment size	Reference
<i>M. enterolobii</i>	Me-F	AAC TTT TGT GAA AGT GCC GCT	250 bp	Long <i>et al.</i> (2006)
	Me-R	TCA GTT CAG GCA GGA TCA ACC		
<i>M. incognita</i>	inc_K14F	GGG ATG TGT AAA TGC TCC TG	399 bp	Zijlstra <i>et al.</i> (2000)
	inc_K14R	CCC GCT ACA CCC TCA ACT TC		
<i>M. javanica</i>	OPA_01Fjav	GGT GCG CGA TTG AAC TGA GC	700 bp	Zijlstra <i>et al.</i> (2000)
	OPA_01Rjav	CAG GCC CTT CAG TGG AAC TAT AC		
<i>M. arenaria</i>	OPA_12Fare	TCG GCG ATA GAG GTA AAT GAC	420 bp	Zijlstra <i>et al.</i> (2000)
	OPA_12Rare	TCG GCG ATA GAC ACT ACA ACT		

Table 2.2b: Polymerase chain reaction (PCR) amplification profiles used with the species-specific primers for identification of *Meloidogyne arenaria*, *M. enterolobii*, *M. incognita* and *M. javanica*. (Zijlstra *et al.*, 2000; Long *et al.*, 2006).

Primer pair	Denaturation temperature and time	Cycle	Denaturation temperature and time	Annealing temperature and time	Extension temperature and time	Final extension temperature and time
Me-F/Me-R	94 °C, 2 min	35	94 °C, 30 s	64 °C, 30 s	72 °C, 1 min	72 °C, 5 min
inc_K14F/inc_K14R	94 °C, 5 min	35	94 °C, 30 s	55 °C, 45 s	72 °C, 2 min	72 °C, 10 min
OPA_01Fjav/OPA_01Rjav	94 °C, 2 min	45	94 °C, 30 s	64 °C, 30 s	72 °C, 1 min	72 °C, 5 min
OPA_12Fare/OPA_12Rare	94 °C, 2 min	35	94 °C, 30 s	64 °C, 30 s	72 °C, 1 min	72 °C, 5 min

2.3 Data analyses

Prominence values (PV) were used for the study to determine both the occurrence frequency and mean population densities of plant-parasitic (genera and/or species) and beneficial nematodes (genera or family levels) (Fourie *et al.*, 2001a; De Waele and Jordaan, 1988a). This parameter serves as an index to categorize nematode genera and/or species (in terms of their predominance). Hence, high PV indicates which nematode genera/species/families were predominant in root and soil samples obtained from each field, while low PV indicates nematode genera/species of minor importance. The equation for calculating PV is as follows:

$$\text{Prominence values} = (\text{population density} \times \sqrt{\text{frequency of occurrence}} / 10)$$

- Population density:

$$= \frac{\text{total number of a nematode genus or species present at each site for each region}}{\text{number of sites on which the nematode genus or species occurred per region}}$$

- Frequency of

$$\text{occurrence} = \frac{\text{number of sites on which the nematode genus or species occurred}}{\text{number of sites sampled}} \times 100$$

Ultimately, the data were pooled and the PV of each family, genus or species were also calculated per province using the same equation and the PV values were created using Microsoft Excel 365.

Nematode community data were analysed using the Nematode Indicator Joint Analysis (NINJA) Online Tool (Sieriebriennikov *et al.*, 2014). The Nematode-based indices (NBIs) calculated included the Basal index (BI), Channel index (CI), Enrichment index (EI), Structure index (SI), and Maturity Index 2-5 (MI 2-5), along with nematode metabolic footprints (MNF) consisting of the Bacterivore, Enrichment, Fungivore, Structure, Herbivore, Omnivore, and Predator footprints. The NBIs were used for soil ecosystem restoration. Multivariate analyses were also done to determine whether relationships existed among nematodes and the selected soil physical and chemical parameters (Gruzdeva *et al.*, 2007). This was done by a redundancy analysis (RDA) and illustrated on biplots using Canoco 5 Software package. Data for each recorded field sampled during the two surveys were subjected to Analysis of Variance (ANOVA) and finally to Factorial ANOVA using (Statistica, Version 14.0.0.15; www.statsoft.com) to determine whether a significant interaction ($P < 0.05$) existed between the two surveys with *Meloidogyne*, *Rotylenchulus*, *Pratylenchus* and *Dolichodoridae* as the main factor. Nematode data were $\log(x + 1)$ transformed before statistical analysis to reduce variation (Ribeiro-Oliveira *et al.*, 2018).

CHAPTER 3: RESULTS – IDENTIFICATION OF *MELOIDOGYNE* SPECIES

3.1 Introduction

According to Ghaderi and Karssen (2020), approximately 105 species of *Meloidogyne* Goeldi, 1892 (root-knot nematodes) have recently been identified and this number can increase annually with the continuous discovery of new species (Blok and Powers, 2009). Hence, accurate identification of root-knot nematodes species is important for developing successful and more importantly sustainable management strategies to reduce losses to grain crops in SA for highly pathogenic species (Abebe *et al.*, 2011). Traditionally, morphological characteristics of mature females, such as perineal patterns, have been used as the main tool to distinguish between species (Jepson, 1987). However, using this approach only is often insufficient as various species share similar traits, while high intra- and interspecies variation also occur. For example, perineal-patterns of *Meloidogyne enterolobii* Yang and Eisenback, 1983 are rounded or squared with high dorsal arches, which is similar to that of *Meloidogyne incognita* (Kofoid and White, 1919) Chitwood, 1949 (Rashidifard *et al.*, 2019b; Karssen *et al.*, 2013). Furthermore, most morphometric traits of second-stage juveniles (J2) and males of these two species overlap (Villar-Luna *et al.*, 2016; Brito *et al.*, 2004). Thus, it is crucial to use all the different morphological and morphometric characteristics, and additionally develop more specific biochemical and/or molecular techniques to distinguish between, and accurately identify *Meloidogyne* spp. (dos Santos *et al.*, 2019).

Since the differences concerning the morphological traits of *Meloidogyne* spp. are in many cases very small, and certain features are influenced by the host plant as well as a range of environmental factors (Jepson, 1987), this study aimed to distinguish the *Meloidogyne* spp. that occurred in fields of 16 grain producers in SA from each other, using sequence characterized amplified region-based polymerase chain reaction (SCAR-PCR) (Zijlstra *et al.*, 2000) to complement morphological identification using oesophageal and perineal-pattern characteristics.

Meloidogyne spp. were only identified from fields where sufficient females and/or eggs and second-stage juveniles (J2) were obtained from infected roots of grain crops during extraction for counting purposes, and deoxyribonucleic acid (DNA) could be extracted. For this reason, identification of *Meloidogyne* spp. could be done from nine fields (see Table 3.1).

3.2 Morphological identification using oesophageal and perineal-pattern characteristics

Focusing on oesophageal and perineal-pattern morphology (see Paragraph 2.2.3.1.2), three *Meloidogyne* spp. were identified from the 18 fields, namely *Meloidogyne arenaria* (Neal, 1889) Cobb, 1890, *M. incognita* and *Meloidogyne javanica* (Treub) Chitwood, 1949 (Table 3.1; Figs. 3.1 – 3.3). *Meloidogyne incognita* dominated by being present in roots of grain crops from 14 fields, followed by *M. javanica* were identified in 11 fields, while *M. arenaria* were present in three fields. Mixtures of three species occurred as: *M. arenaria*, *M. incognita* and *M. javanica* present in two fields (Table 3.1); *M. incognita* and *M. javanica* occurred as mixed communities in six fields; and *M. arenaria* and *M. javanica* were present as mixed communities in one field.

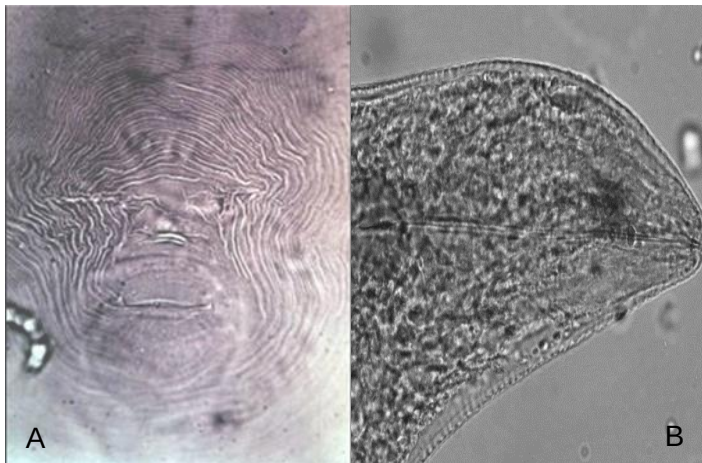


Figure 3.1: A perineal-pattern of a *Meloidogyne arenaria* mature female (A) and its oesophagus (Photo: Nemaplex) (B) using at 100x magnification using a Nikon Eclipse 50i light microscope (Photo: Bongiwé Mhlongo, NWU).

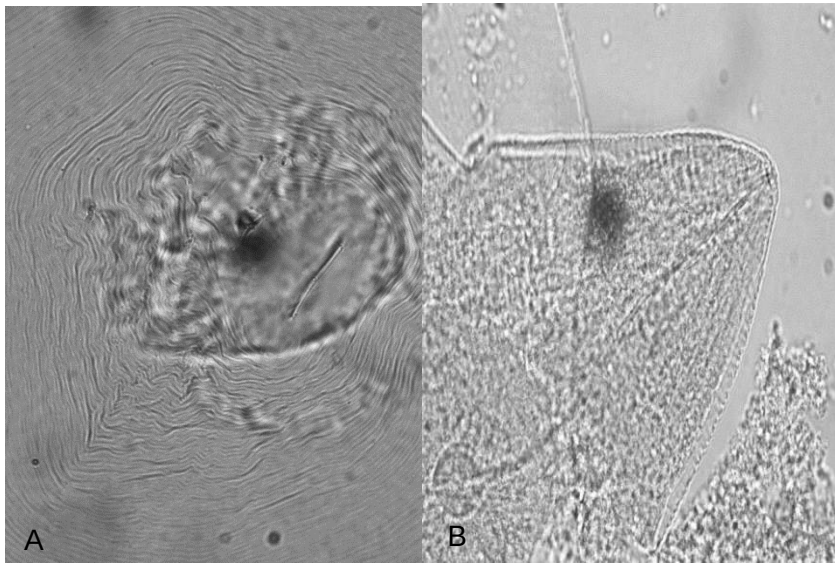


Figure 3.2: A perineal-pattern of a *Meloidogyne incognita* mature female (A) and its oesophagus (B) using at 100x magnification using a Nikon Eclipse 50i light microscope (Photo: Bongiwe Mhlongo, NWU).

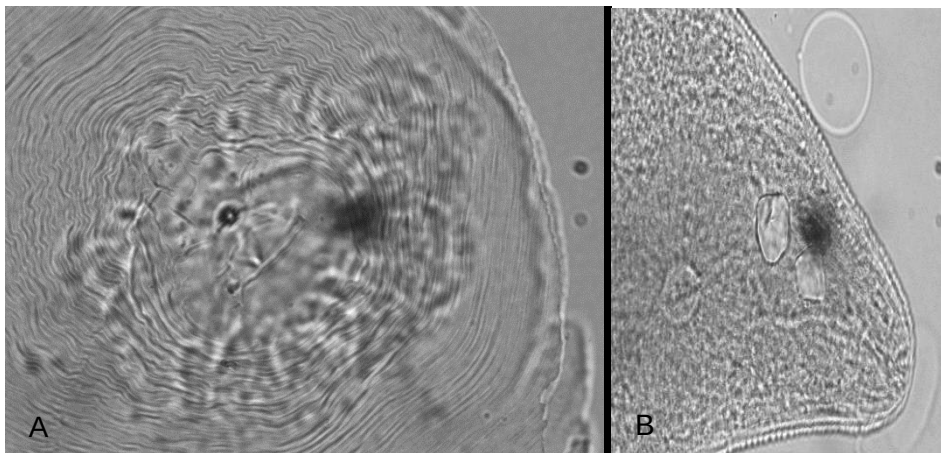


Figure 3.3: A perineal-pattern of a *Meloidogyne javanica* mature female (A) and its oesophagus (B) using at 100x magnification using a Nikon Eclipse 50i light microscope (Photo: Bongiwe Mhlongo, NWU).

3.3 Molecular identification using sequence characterized amplified region-based polymerase chain reaction (SCAR-PCR).

Using the SCAR-PCR technique (Chapter 2, Paragraph 2.2.3.1.3), four *Meloidogyne* spp. were identified from the 18 fields (Table 3.1). *Meloidogyne incognita* was predominant and present in 13 of the fields (Fig. 3.5), followed by *M. javanica* being present in 10 fields (Fig.

3.6), and *M. arenaria* present in six fields (Fig. 3.7); *M. enterolobii* was not detected in any of the fields sampled (Fig. 3.8).

Meloidogyne incognita female DNA resulted in the 399 bp SCAR fragment being amplified (Zijlstra *et al.*, 2000) for 13 fields of the fields and agreed with the same size DNA band expressed for the *M. incognita* species-specific primer (Fig. 3.5).

Meloidogyne javanica female DNA resulted in the 700 bp SCAR fragment being amplified (Zijlstra *et al.*, 2000) for 10 of the fields and agreed with the same size DNA band amplified for the *M. javanica* species-specific marker (Fig. 3.6).

Meloidogyne arenaria female DNA resulted in the 420 bp SCAR fragment being amplified (Zijlstra *et al.*, 2000) in six of the fields which corresponded to that of the *M. arenaria* species-specific primer (Fig. 3.7).

Nine of the 18 fields contained mixed *Meloidogyne* spp. communities: a mixture of three of the species (*M. arenaria*, *M. incognita* and *M. javanica*) was present in two fields, while *M. incognita* and *M. javanica* occurred as mixed communities in three of the fields. *Meloidogyne arenaria* and *M. javanica* occurred together in three fields and *M. arenaria* and *M. incognita* were present in one field.

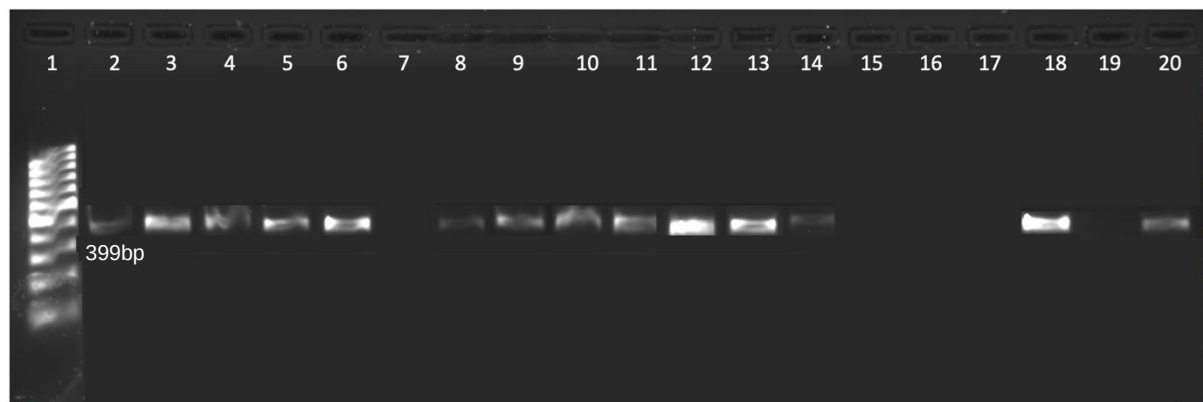


Figure 3.5: Photograph of agarose gel with DNA amplification products of *Meloidogyne incognita* (*Mi*) females obtained from 13 of the 18 fields sampled using SCAR-PCR (Field ID: 3=1HS; 4=2DM; 5=4CS_1; 6=4CS_2; 7=5JM; 8=7WM; 9=JB; 10=10MG; 11=11IM; 12=13PvZ; 13=DV; 14=16SJ; 15=17JB_1; 16=17JB_2; 17=17JB_3; 18=18JF; 19=20NR; 20=22PZ). 1=1KB ladder; 2=*Mi* standard at 399bp).

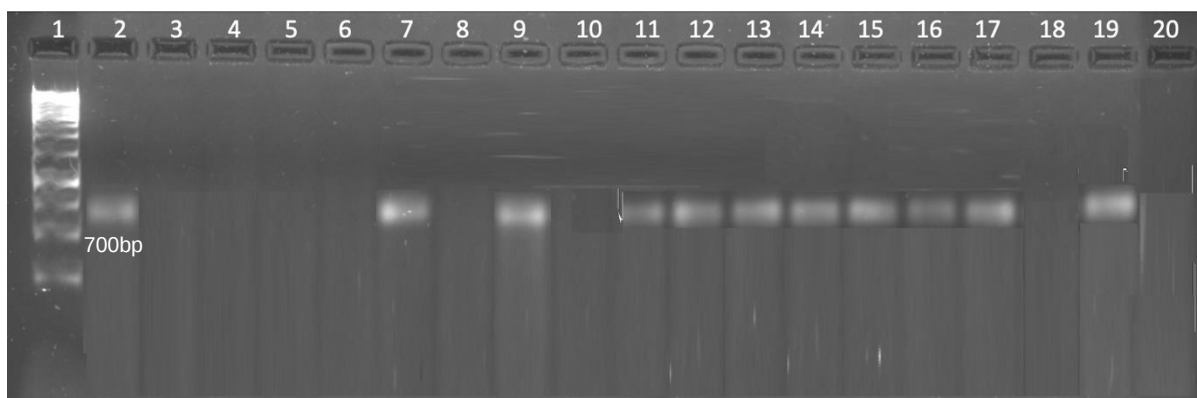


Figure 3.6: Photograph of agarose gel with DNA amplification products of *Meloidogyne javanica* (*Mj*) females obtained from 10 of the 18 fields sampled using SCAR-PCR (Field ID: 3=1HS; 4=2DM; 5=4CS_1; 6=4CS_2; 7=5JM; 8=7WM; 9=JB; 10=10MG; 11=11IM; 12=13PvZ; 13=DV; 14=16SJ; 15=17JB_1; 16=17JB_2; 17=17JB_3; 18=18JF; 19=20NR; 20=22PZ). 1=1KB ladder; 2=*Mj* standard at 700bp.

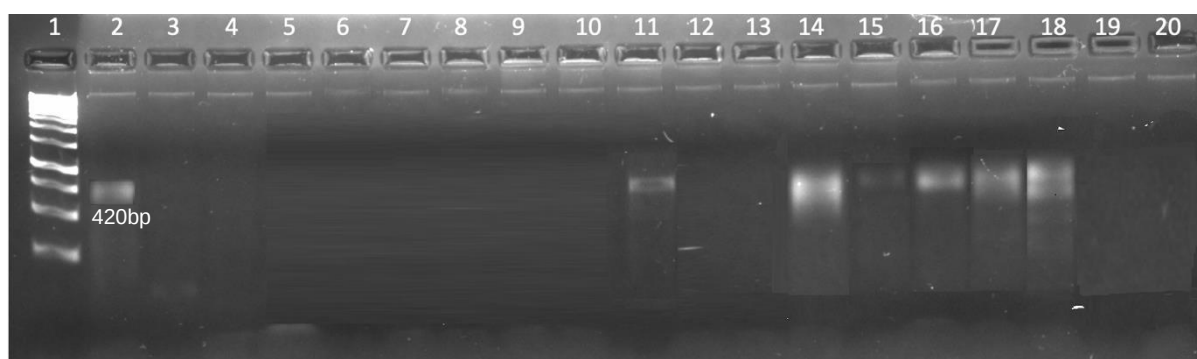


Figure 3.7: Photograph of agarose gel with DNA amplification products of *Meloidogyne arenaria* (*Ma*) females obtained from six of the 18 fields sampled using SCAR-PCR (Field ID: 3=1HS; 4=2DM; 5=4CS_1; 6=4CS_2; 7=5JM; 8=7WM; 9=JB; 10=10MG; 11=11IM; 12=13PvZ; 13=DV; 14=16SJ; 15=17JB_1; 16=17JB_2; 17=17JB_3; 18=18JF; 19=20NR; 20=22PZ). 1=1KB ladder; 2=*Ma* standard at 420bp.

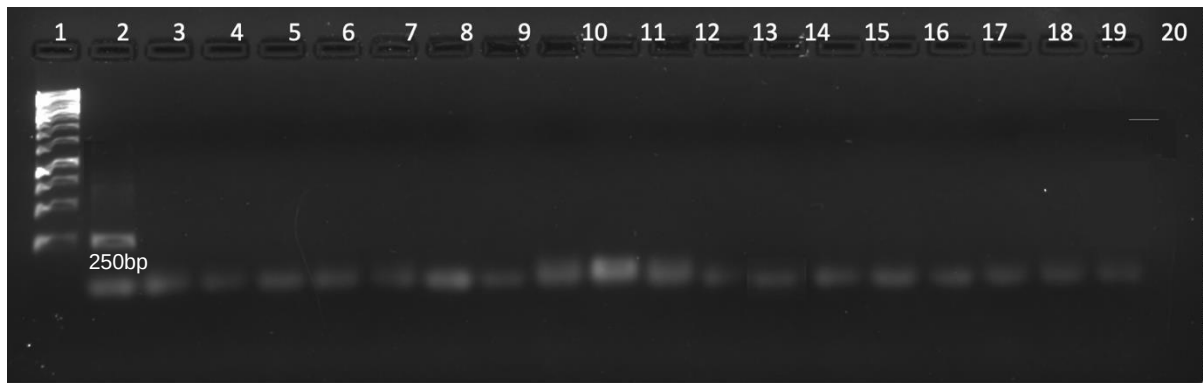


Figure 3.8: Photograph of agarose gel with no DNA amplification products of *Meloidogyne enterolobii* females obtained from the 18 fields from 2020/21 growing using SCAR-PCR (Field ID: 3=1HS; 4=2DM; 5=4CS_1; 6=4CS_2; 7=5JM; 8=7WM; 9=JB; 10=10MG; 11=11IM; 12=13PvZ; 13=DV; 14=16SJ; 15=17JB_1; 16=17JB_2; 17=17JB_3; 18=18JF; 19=20NR; 20=22PZ). 1=1KB ladder; 2=Me standard at 250bp.

Table 3.1: Identification of *Meloidogyne* spp. present in 18 fields of grain producers during the 2020 and 2021 growing seasons using species-specific SCAR-PCR primers and perineal-patterns.

Sample ID	Field, province	Crop 2020/21	¹ Ma	² Me	³ Mi	⁴ Mj	¹ Ma	² Me	³ Mi	⁴ Mj
Molecular (SCAR-PCR method)							Morphology (perineal patterns)			
1SH	Carletonville, Gauteng	Wheat/ soybean	-	-	Yes	-	-	-	Yes	-
2DM	Lichtenburg, North West	Maize	-	-	Yes	-	-	-	Yes	-
4CS_1	Hoopstad, Free Sate	Maize	-	-	Yes	-	-	-	Yes	Yes
4CS_2	Hoopstad, Free Sate	Maize	-	-	Yes	-	-	-	Yes	Yes
5JM	Bothaville, Free State	Maize	-	-	-	Yes	-	-	Yes	Yes
7WM	Hennenman, Free State	Sunflower/Maize	-	-	Yes	-	-	-	Yes	-
8JB	Hoopstad, Free State	Soybean/ wheat	-	-	Yes	Yes	-	-	Yes	-
10MG	Hennenman, Free State	Maize	-	-	Yes	-	-	-	Yes	-
11IM	Hoopstad, Free State	Sunflower	Yes	-	Yes	Yes	Yes	-	Yes	Yes
13PvZ	Bothaville, Free State	Maize	-	-	Yes	Yes	-	-	Yes	Yes
14DV	Bothaville, Free State	Maize	-	-	Yes	Yes	-	-	Yes	Yes
16SJ	Bothaville, Free State	Maize	Yes	-	Yes	Yes	Yes	-	Yes	Yes
17JB_1	Bothaville, Free State	Wheat/Maize/soybean	Yes	-	-	Yes	-	-	-	Yes
17JB_2	Bothaville, Free State	Wheat/Maize/soybean	Yes	-	-	Yes	Yes	-	-	Yes
17JB_3	Bothaville, Free State	Wheat/Maize/soybean	Yes	-	-	Yes	-	-	-	Yes
18JF	Bothaville, Free State	Maize	Yes	-	Yes	-	-	-	Yes	-
20NR	Ottosdal, North West	Sunflower/Maize	-	-	-	Yes	-	-	-	Yes
22PZ	Vrede, Free State	Maize/Soybean	-	-	Yes	-	-	-	Yes	-

¹Ma = *M. arenaria*: 420 bp; ²Me = *M. enterolobii*: 250 bp; ³Mi = *M. incognita*: 399 bp; ⁴Mj = *M. javanica*: 700bp.

3.4 Discussion and conclusion

Three *Meloidogyne* spp. (*M. arenaria*, *M. incognita* and *M. javanica*) were successfully identified using the classical morphology approach mainly based on perineal-pattern characteristics as well as the DNA-based SCAR-PCR technique. The three species identified represent the three most important ones that parasitize grain crops in SA, namely *M. arenaria*, *M. incognita* and *M. javanica* (Marais *et al.*, 2017; Jones *et al.*, 2013; Kleynhans *et al.*, 1996; Kleynhans, 1991). *M. enterolobii*, recently recorded from the Mpumalanga Highveld maize-production area of South Africa (Pretorius, 2018) as well as from the Free State and North-West provinces (Qwabe, 2023), was not identified as a result of this study.

While the results of the perineal-pattern approach supported the molecular identification of the three species, it is acknowledged that it was difficult to distinguish the perineal-patterns of *M. enterolobii* and *M. incognita* from each other. It is hence agreed with, Rashidifard *et al.* (2019b, c), Visagie *et al.* (2018), Carneiro *et al.* (2017) and Villar-Luna *et al.* (2016) that using the perineal-pattern approach only, is challenging and may lead to misidentification of cryptic species, even by experienced taxonomists. Therefore, combining both classical, molecular and even biochemical (isozyme esterase) approaches should be done to ensure accurate identification of *Meloidogyne* spp. Identification of *M. arenaria* in maize-based production areas of SA confirms previous reports by Pretorius (2018), Agenbag (2016), Riekert and Henshaw (1998) and Kleynhans *et al.* (1996) that this species occurred in such areas but in low rate as compared to the other predominant species.

Identification of mixed communities of *Meloidogyne* spp. agrees with published reports. The presence of two, three and/or four *Meloidogyne* spp. occurring in mixed communities in local grain fields has been confirmed recently by Fourie *et al.* (2017), Mc Donald *et al.*, (2017), Rashidifard *et al.* (2019b, c), Visagie *et al.* (2018) and Pretorius (2018), and also earlier by Riekert and Henshaw (1998). This agrees with a report by a renowned *Meloidogyne* spp. taxonomist, Karssen *et al.* (2013), that species of this genus often are found in mixed communities across the globe.

The predominant root-knot nematodes species identified in this study, namely *M. incognita* and *M. javanica*, are known to cause problems in local grain crops since most of these crops, e.g., maize (*Zea mays* L.), soybean (*Glycine max* L.), sunflower (*Helianthus annuus* L.) and wheat (*Triticum aestivum* L.) are known to host them (Fourie *et al.*, 2017; Mc Donald *et al.*, 2017). Dedicated and well-planned management strategies can only be employed once the species' identity is known (Rashidifard *et al.*, 2019a, b, c; Agenbag, 2016; McSorley, 2011 and Xiang *et al.*, 2010). In addition, planting resistant and/or tolerant genotypes will be an option to reduce root-knot nematodes damage in grain crops, while using the best complementary cropping practices to reduce the population densities of

Meloidogyne spp. below damage threshold levels. Ultimately, the application of these strategies is dependent on a good understanding and knowledge of the taxonomy and biology of the target *Meloidogyne* spp. (Ahmed *et al.*, 2016).

CHAPTER 4: RESULTS: NEMATODE SURVEY (PLANT-PARASITIC AND BENEFICIAL NEMATODES)

4.1 Introduction

The three grain crops maize (*Zea mays* L.), soybean (*Glycine max* (L.) Merr.) and sunflower (*Helianthus annuus* L.) are locally mainly parasitized and damaged by root-knot nematodes, *Meloidogyne* Goeldi, 1892, and lesion nematodes, *Pratylenchus* Filipjev, 1936 (Mc Donald *et al.*, 2017; Fourie *et al.*, 2017). Concerning wheat (*Triticum aestivum* L.), the predominant plant-parasitic nematode genus identified to parasitise the crop locally is lesion nematodes (Mc Donald *et al.*, 2017). Considering the progress made in terms of developing new grain crop varieties, improved crop production practices, more information becoming available on the effect that climate change has on crop production and the updated information about diseases and pests associated with crops, it is suggested that regular nematode-crop surveys or audits be done. This will enable researchers to monitor whether shifts in terms of plant-parasitic nematodes abundance and community composition occur. Most of all, if the occurrence of new or more pathogenic species becomes apparent that can compromise sustainable crop production and threaten food security researchers and producers will be able to consider appropriate management strategies.

Ultimately, grain producers requested a nematode survey under the auspices of Grain SA to be done in their fields since they experienced problems with plant-parasitic nematodes and associated microbe-induced diseases. As a result, maize, soybean, sunflower and wheat fields of concerned farmers in the Free State, Gauteng and North-West provinces were sampled during two consecutive summer growing seasons, 2020 (referred to from hereon as Survey 1) and 2021 (referred to from hereon as Survey 2), as part of nematode surveys. As indicated in more detail in Chapter 2 (Methodology or Materials and Methods) the data were analysed by calculating the prominence values (PV) (see Paragraph 2.1.5; De Waele and Jordaan, 1988a; Fourie *et al.*, 2001a). Furthermore, population density data for the three predominant plant-parasitic nematodes for Survey 1 and Survey 2 were subjected to Repeated Measures ANOVA (TIBCO Statistica® Version 13.3; <https://docs.tibco.com/products/tibco-statistica-14-0-1>) for comparison. In addition, selected nematode-based indices (NBIs) were calculated for Survey 2 by subjecting the community data for beneficial and plant-parasitic nematodes to the Nematode Indicator Joint Analysis Online Tool (NINJA: Sieriebriennikov *et al.*, 2014). The NBIs calculated for each field were also subjected to Factorial Analyses of Variance (ANOVA) using the TIBCO Statistica™ Version 14.0.0.15 Software Package (<https://www.statistica.com>). Ultimately, associations between plant-parasitic nematodes and beneficial nematodes and selected abiotic

parameters and the treatments (fields of producers) were investigated using the Canoco 5 Software Package (<http://www.canoco5.com/>).

4.2 Results of nematodes data

4.2.1 Roots (50 g)

Life stage individuals of two sessile, endoparasitic nematode genera were identified from 50 g root samples during both surveys, namely root-knot nematodes *Meloidogyne* Goeldi, 1892 and spiral nematodes belonging to the genus *Rotylenchulus* Linford and Oliveira, 1940 (Table 4.1a and b).

Root-knot nematode eggs and second-stage juveniles (J2) were present in 50 g root samples obtained from 17 fields during both Surveys 1 and 2, with PV ranging from zero to 8915 and from zero to 1270 respectively (Table 4.1a). The MPD for this genus ranged from zero to 9766 and from zero to 1494, while the %FO ranged from zero to 100% respectively for the two surveys.

A significant interaction was evident for root-knot nematode abundance/50 g roots between the two surveys, indicating that some of the localities had significantly different numbers of this genus (Annexure: Table A4.1a; Fig 4.1a). These fields were 2DM (North West province; Lichtenburg area), 4CS (Free State province; Hoopstad area) and 8JB (Free State province; Hoopstad area) had significantly higher root-knot nematode abundance during survey 1 compared to survey 2 (indicated in green arrows in Fig. 4.1a). No significant differences in root-knot nematode abundance were evident between the two surveys for the other 15 fields.

Table 4.1a: Frequency of occurrence (FO), mean population density (MPD) and prominence values (PV) for *Meloidogyne* per 50 g roots of grain crops sampled from 18 fields situated in the Gauteng, North-West and Free State provinces of South Africa, during the 2020 (Survey 1) and 2021 (Survey 2) summer growing seasons.

Field no	Field code	Province	Crop sequence 2020/2021	2020 (Survey 1)			2021 (Survey 2)		
				FO	MPD	PV	FO	MPD	PV
1	1SH	Gauteng	Wheat/Soybean	100	940	940	50	82	58
2	2DM	North West	Sunflower/Maize	100	4825	4825	67	24	19
3	4CS	Free State	Maize/Maize	100	8838	8838	50	4	3
4	4CS_2	Free State	Maize/Maize	67	161	131	33	29	17
5	5JM	Free State	Maize/Maize	100	388	388	83	1391	1270
6	7WM	Free State	Sunflower/maize	83	9766	8915	17	17	7
7	8JB	Free State	Wheat/Soybean	0	0	0	50	17	12
8	10MG	Free State	Maize/Maize	67	707	577	83	548	501
9	11IM	Free State	Sunflower/Sunflower	100	2756	2756	100	239	239
10	13PvZ	Free State	Maize/Maize	100	713	713	100	929	929
11	14DV	Free State	Maize/Maize	100	1162	1162	83	400	365
12	16SJ	Free State	Maize/Maize	100	471	471	83	290	265
13	17JB_1	Free State	Maize/Soybean	67	2674	2184	67	45	37
14	17JB_2	Free State	Soybean/Maize	100	436	436	33	32	18
15	17JB_3	Free State	Wheat/Maize	100	234	234	67	82	67
16	18JF	Free State	Maize/Maize	83	647	590	67	1494	1220
17	20NR	North West	Sunflower/Maize	83	619	565	83	30	27
18	22PZ	Free State	Maize/soybean	17	1146	468	0	0	0

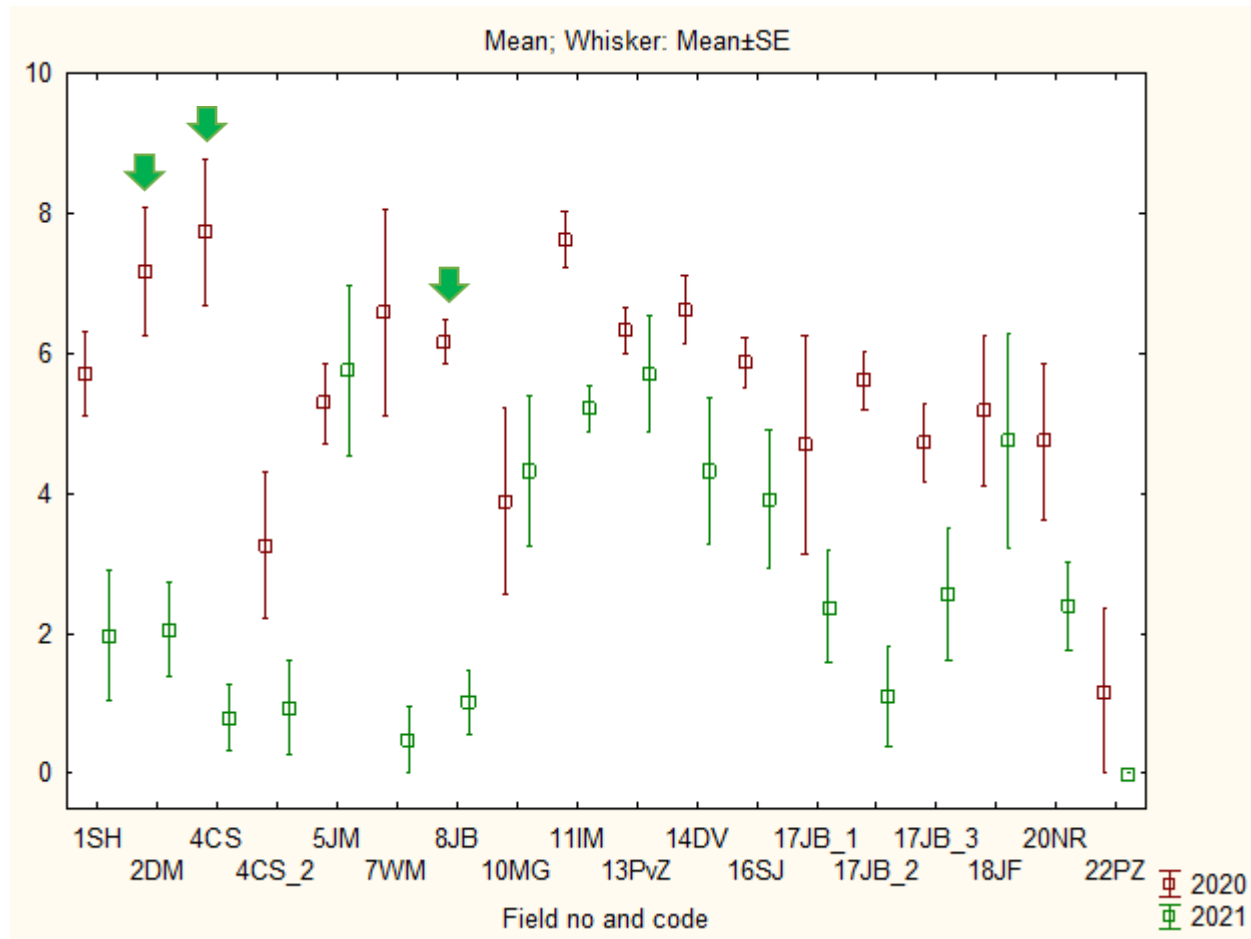


Figure 4.1a: *Meloidogyne* spp. numbers/50 g roots sampled at farms of 18 grain producers during two consecutive growing seasons (2020 and 2021). The green arrows show significant differences in root-knot nematode numbers during the two growing seasons for those localities.

Rotylenchulus were present in 50 g root samples obtained from five fields during both Surveys 1 and 2, with PV ranging from zero to 978 and from zero to 447 respectively. (Table 4.1b). The MPD for this genus ranged from zero to 2348 and from zero to 775, while the %FO ranged from zero to 100% for both surveys.

A significant interaction was evident for *Rotylenchulus* abundance/50 g roots between the two surveys, indicating that some of the localities had significantly different numbers of this genus (Annexure: Table A4.1b; Fig 4.1b). These fields were 2DM (North West province; Lichtenburg area), 7WM (Free State province; Henneman area), 8JB (Free State province; Hoopstad area) and 22PZ (Free State province; Vrede area) (indicated with green arrows in Fig. 4.1b) with significantly higher *Rotylenchulus* abundance during survey 1 compared to survey 2. No significant differences in *Rotylenchulus* abundance were evident between the two surveys for the other 14 fields.

Table 4.1b: Frequency of occurrence (FO), mean population density (MPD) and prominence values (PV) for *Rotylenchulus* per 50 g roots of grain crops sampled from 18 fields situated in the Gauteng, North-West and Free State provinces of South Africa, during the 2020 (Survey 1) and 2021 (Survey 2) summer growing seasons.

Field no	Field code	Province	Crop sequence 2020/2021	2020 (Survey 1)			2021 (Survey 2)		
				FO	MPD	PV	FO	MPD	PV
1	1SH	Gauteng	Wheat/Soybean	0	0	0	0	0	0
2	2DM	North West	Sunflower/Maize	0	0	0	100	59	59
3	4CS	Free State	Maize/Maize	0	0	0	0	0	0
4	4CS_2	Free State	Maize/Maize	17	50	20	0	0	0
5	5JM	Free State	Maize/Maize	17	47	19	50	10	7
6	7WM	Free State	Sunflower/maize	0	0	0	83	93	85
7	8JB	Free State	Wheat/Soybean	0	0	0	0	0	0
8	10MG	Free State	Maize/Maize	100	412	412	83	198	181
9	11IM	Free State	Sunflower/Sunflower	0	0	0	0	0	0
10	13PvZ	Free State	Maize/Maize	0	0	0	0	0	0
11	14DV	Free State	Maize/Maize	0	0	0	0	0	0
12	16SJ	Free State	Maize/Maize	0	0	0	0	0	0
13	17JB_1	Free State	Maize/Soybean	17	2348	959	33	775	447
14	17JB_2	Free State	Soybean/Maize	0	0	0	0	0	0
15	17JB_3	Free State	Wheat/Maize	0	0	0	0	0	0
16	18JF	Free State	Maize/Maize	0	0	0	0	0	0
17	20NR	North West	Sunflower/Maize	0	0	0	0	0	0
18	22PZ	Free State	Maize/soybean	67	1198	978	0	0	0

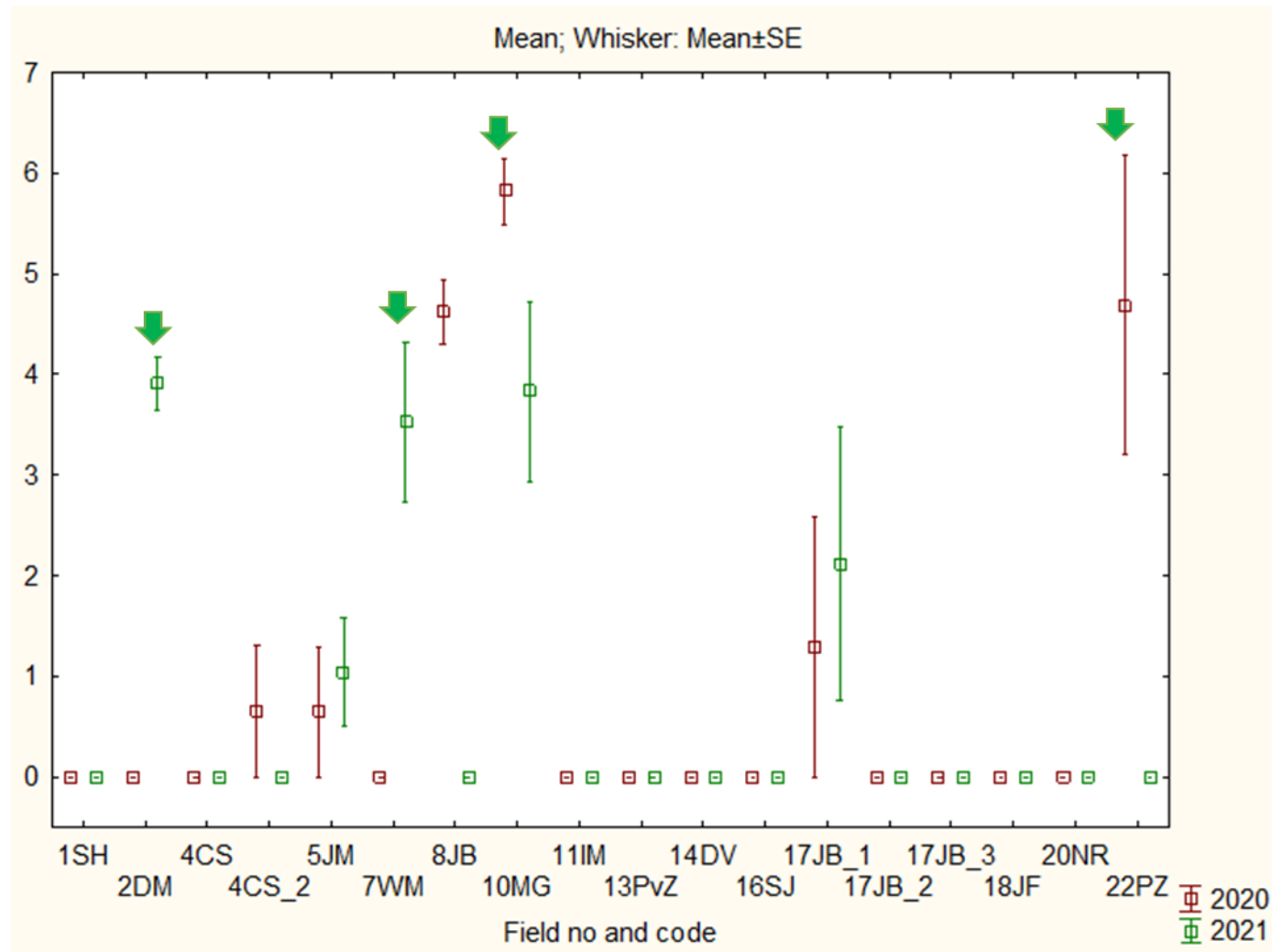


Figure 4.1b: *Rotylenchulus* spp. numbers/50 g roots sampled at farms of 18 grain producers during two consecutive growing seasons (2020 and 2021). The green arrows show significant difference in root-knot nematode numbers during the two growing seasons for those localities.

4.2.2 Roots (5 g)

Meloidogyne eggs and J2 were present in 5 g root samples obtained from 16 and 17 fields respectively during Surveys 1 and 2, with PV ranging from zero to 164 and from zero to 76 respectively (Table 4.2a). The MPD for this genus ranged from zero to 183 and zero to 76, while the %FO ranged from zero to 100% for both surveys.

A significant interaction was evident for root-knot nematode abundance/5 g roots between the two surveys, indicating that some of the localities had significantly different numbers of this genus (Annexure: Table A4.2a; Fig 4.2a). Fields 4CS (Free State province; Hoopstad area) for example had significantly higher root-knot nematode abundance during survey 1 compared to survey 2. For 22PZ (Free State province; Vrede area) the opposite was true since a higher root-knot nematode abundance was evident in 2021 compared to 2020 (indicated with green arrows in Fig. 4.2a). No significant differences in root-knot nematode abundance between the two surveys were evident for the other 16 fields.

Table 4.2a: Frequency of occurrence (FO), mean population density (MPD) and prominence values (PV) of *Meloidogyne* spp. per 5 g roots of grain crops sampled from 18 fields situated in the Gauteng, North-West and Free State provinces of South Africa, during the 2020 (Survey 1) and 2021(Survey 2) summer growing seasons.

Field no	Field code	Province	Crop sequence 2020/2021	2020 (Survey 1)			2021 (Survey 2)		
				FO	MPD	PV	FO	MPD	PV
1	1SH	Gauteng	Wheat/Soybean	33	31	18	100	74	74
2	2DM	North West	Sunflower/Maize	33	29	17	100	31	31
3	4CS_1	Free State	Maize/Maize	100	65	65	25	8	4
4	4CS_2	Free State	Maize/Maize	100	21	21	0	0	0
5	5JM	Free State	Maize/Maize	0	0	0	100	12	12
6	7WM	Free State	Sunflower/maize	50	183	129	83	25	23
7	8JB	Free State	Wheat/Soybean	0	0	0	33	32	18
8	10MG	Free State	Maize/Maize	17	17	7	83	70	64
9	11IM	Free State	Sunflower/Sunflower	100	164	164	100	60	60
10	13PvZ	Free State	Maize/Maize	100	109	109	83	67	61
11	14DV	Free State	Maize/Maize	83	71	65	100	64	64
12	16SJ	Free State	Maize/Maize	100	25	25	100	76	76
13	17JB_1	Free State	Maize/Soybean	17	17	7	100	32	32
14	17JB_2	Free State	Soybean/Maize	33	6	3	50	55	39
15	17JB_3	Free State	Wheat/Maize	17	146	60	100	40	40
16	18JF	Free State	Maize/Maize	17	21	9	100	35	35
17	20NR	North West	Sunflower/Maize	33	25	14	100	18	18
18	22PZ	Free State	Maize/soybean	33	11	6	100	60	60

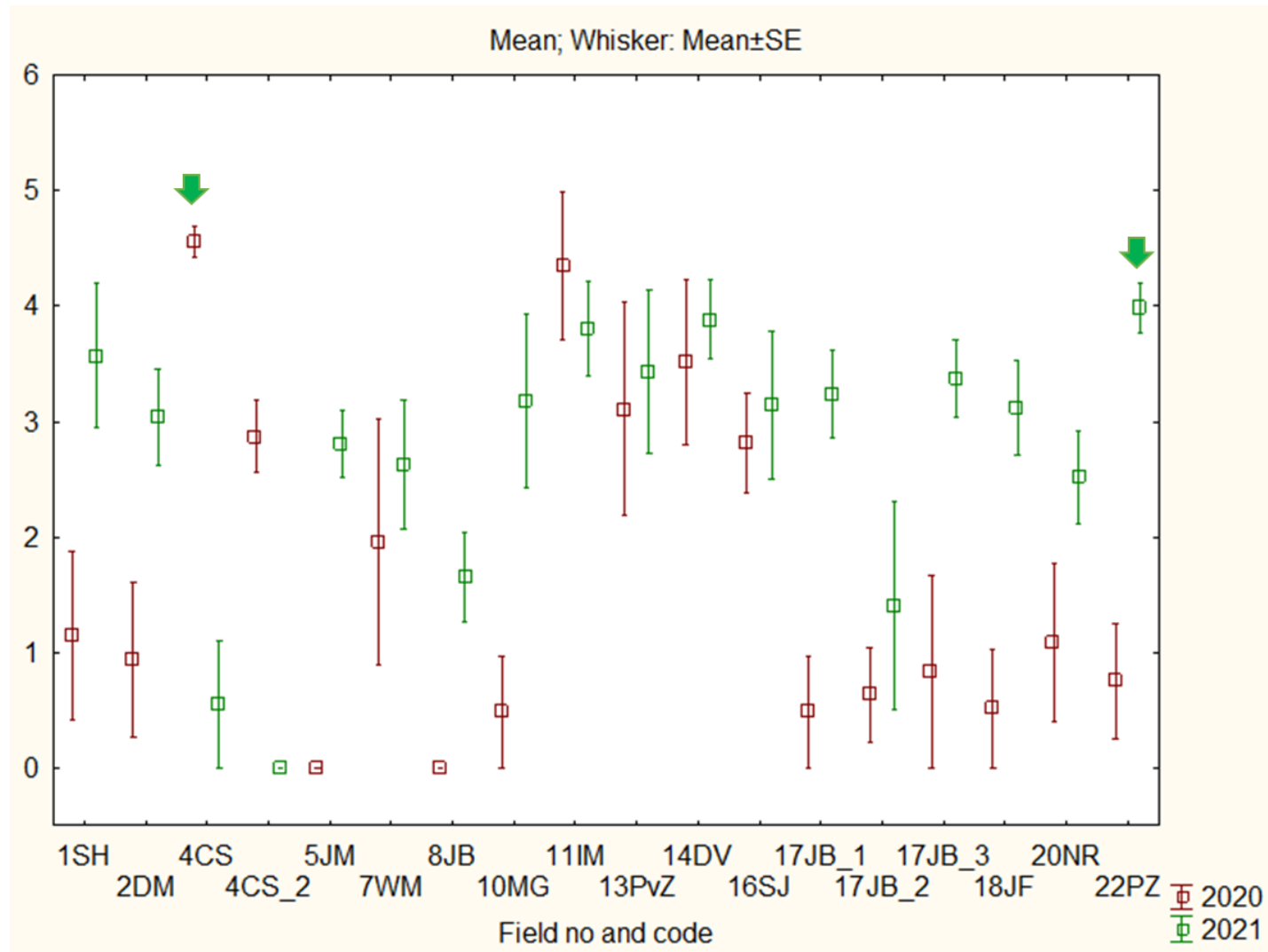


Figure 4.2a: *Meloidogyne* spp. numbers/5 g roots sampled at farms of 18 grain producers during two consecutive growing seasons (2020 and 2021). The green arrows show significant differences in root-knot nematode numbers during the two growing seasons.

Pratylenchus spp. eggs and J2 were present in 5 g root samples obtained from 16 and 18 fields during Surveys 1 and 2, with PV ranging from zero to 169 and from zero to 366 respectively (Table 4.2b). The MPD for this genus ranged from zero to 185 and from zero to 500, while the %FO ranged from zero to 100% for both surveys.

A significant interaction was evident for lesion nematode abundance/5 g roots between the two surveys, indicating that some of the localities had significantly different numbers of this genus (Annexure: Table A4.2b; Fig 4.2b). These fields were 5 JM and 14 DV, both located in the Free State province (Bothaville area), as well as 22PZ (Free State province, Vrede area) with significantly higher lesion nematode abundance during the 2021 survey compared to the 2020 survey (indicated with green arrows in Fig. 4.2b). No significant differences in lesion nematode abundance were evident between the two surveys for the other 15 fields.

Rotylenchulus were present in 5 g root samples obtained from 3 fields during both Surveys 1 and 2, with PV ranging from zero to 60 and zero to 19 respectively. (Table 4.2c). The MPD for this genus ranged from zero to 146 and from zero to 47, while the %FO ranged from zero to 67% and from zero to 83% respectively for the two surveys.

Table 4.2b: Frequency of occurrence (FO), mean population density (MPD) and prominence values (PV) *Pratylenchus* spp. per 5 g roots of grain crops sampled from 18 fields situated in the Gauteng, North-West and Free State provinces of South Africa, during the 2020 (Survey 1) and 2021 (Survey 2) summer growing seasons.

Field no	Field code	Province	Crop sequence 2020/2021	2020 (Survey 1)			2021 (Survey 2)		
				FO	MPD	PV	FO	MPD	PV
1	1SH	Gauteng	Wheat/Soybean	17	50	20	83	2	2
2	2DM	North West	Sunflower/Maize	100	168	168	83	84	76
3	4CS_1	Free State	Maize/Maize	100	34	34	75	423	366
4	4CS_2	Free State	Maize/Maize	83	52	48	100	114	114
5	5JM	Free State	Maize/Maize	0	0	0	100	89	89
6	7WM	Free State	Sunflower/maize	50	4	3	83	83	75
7	8JB	Free State	Wheat/Soybean	0	0	0	33	23	13
8	10MG	Free State	Maize/Maize	83	185	169	83	74	68
9	11IM	Free State	Sunflower/Sunflower	33	15	8	83	14	13
10	13PvZ	Free State	Maize/Maize	100	95	95	100	88	88
11	14DV	Free State	Maize/Maize	50	25	18	100	258	258
12	16SJ	Free State	Maize/Maize	83	60	55	100	72	72
13	17JB_1	Free State	Maize/Soybean	100	38	38	83	21	21
14	17JB_2	Free State	Soybean/Maize	100	132	132	50	500	354
15	17JB_3	Free State	Wheat/Maize	83	78	71	100	119	119
16	18JF	Free State	Maize/Maize	100	93	93	67	55	45
17	20NR	North West	Sunflower/Maize	50	24	17	33	9	5
18	22PZ	Free State	Maize/soybean	17	8	3	100	198	198

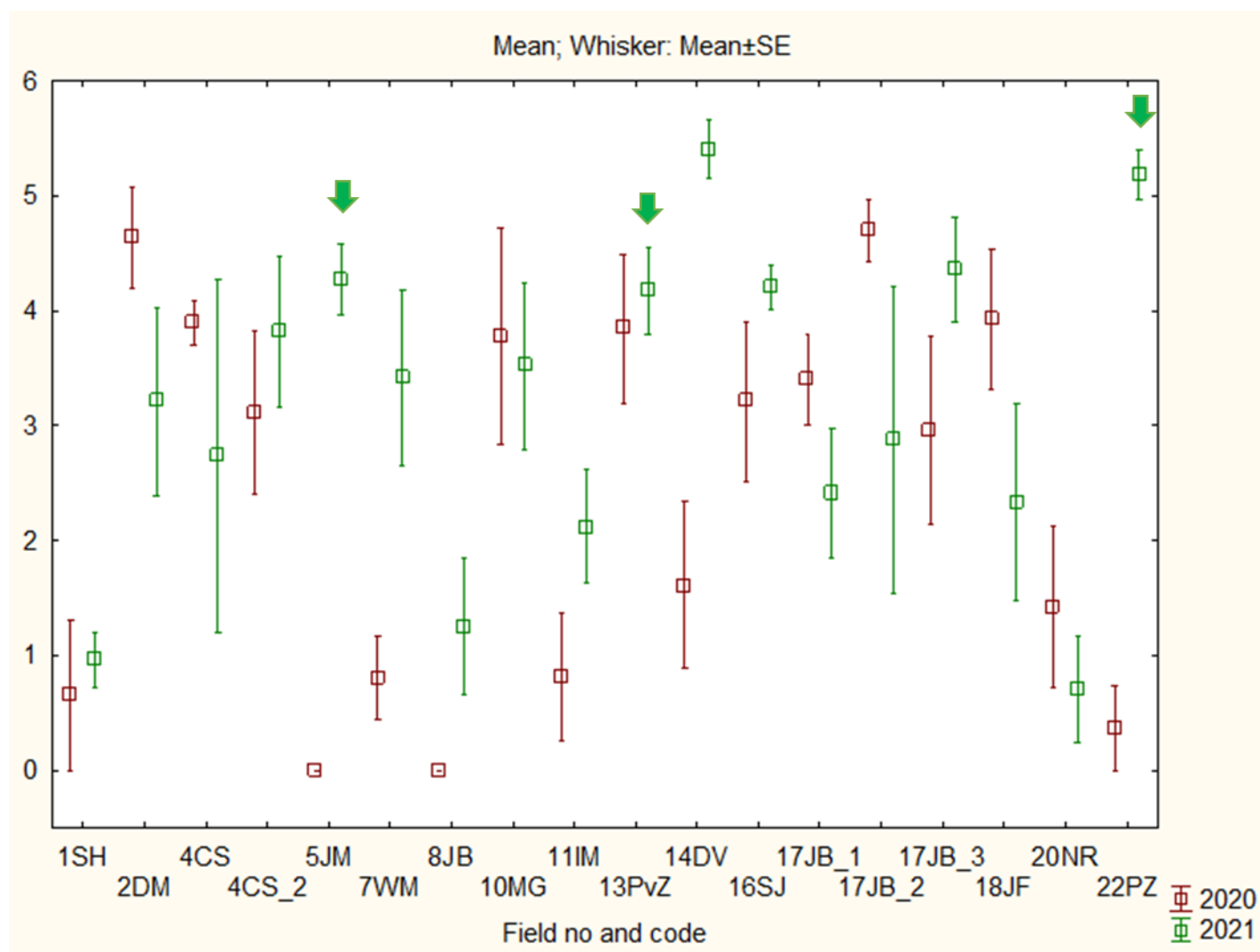


Figure 4.2b: *Pratylenchus* spp. numbers/5 g roots sampled at farms of 18 grain producers during two consecutive growing seasons (2020 and 2021). The green arrows show significant differences in root-knot nematode numbers during the two growing seasons for those localities.

Table 4.2c: Frequency of occurrence (FO), mean population density (MPD) and prominence values (PV) *Rotylenchulus* per 5 g roots of grain crops sampled from 18 fields situated in the Gauteng, North-West and Free State provinces of South Africa, during the 2020 (Survey 1) and 2021 (Survey 2) summer growing seasons.

Field no	Field code	Province	Crop sequence 2020/2021	2020 (Survey 1)			2021 (Survey 2)		
				FO	MPD	PV	FO	MPD	PV
1	1SH	Gauteng	Wheat/Soybean	0	0	0	83	10	9
2	2DM	North West	Sunflower/Maize	0	0	0	0	0	0
3	4CS_1	Free State	Maize/Maize	0	0	0	0	0	0
4	4CS_2	Free State	Maize/Maize	0	0	0	0	0	0
5	5JM	Free State	Maize/Maize	50	13	9	33	3	2
6	7WM	Free State	Sunflower/maize	0	0	0	0	0	0
7	8JB	Free State	Wheat/Soybean	0	0	0	0	0	0
8	10MG	Free State	Maize/Maize	0	0	0	0	0	0
9	11IM	Free State	Sunflower/Sunflower	0	0	0	0	0	0
10	13PvZ	Free State	Maize/Maize	0	0	0	0	0	0
11	14DV	Free State	Maize/Maize	0	0	0	0	0	0
12	16SJ	Free State	Maize/Maize	0	0	0	0	0	0
13	17JB_1	Free State	Maize/Soybean	0	0	0	0	0	0
14	17JB_2	Free State	Soybean/Maize	0	0	0	0	0	0
15	17JB_3	Free State	Wheat/Maize	17	146	60	17	47	19
16	18JF	Free State	Maize/Maize	0	0	0	0	0	0
17	20NR	North West	Sunflower/Maize	0	0	0	0	0	0
18	22PZ	Free State	Maize/soybean	67	39	32	0	0	0

4.2.3 Soil (200g)

4.2.3.1 Plant-parasitic nematodes

Plant-parasitic nematode genera and/or families present in soil samples were as follows in terms of predominance: Dolichodoridae Chitwood, 1950, *Pratylenchus*, Criconematidae Taylor, 1936, Hoplolaimidae Filipjev, 1934, *Meloidogyne*, Trichodoridae Thorne, 1935, *Rotylenchulus*, Longidoridae Thorne, 1935, and *Ditylenchus* Filipjev, 1936 (Tables 4.3a - 4.3j).

Pratylenchus individuals were present in soil samples obtained from 17 and 18 fields during Surveys 1 and 2, respectively (Table 4.3b). The PV for this genus ranged from zero to 380 and from 3 to 548 for the respective surveys and the MPD from zero to 380 and from 4 to 548. The %FO ranged from zero to 100% for Survey 1 and from 17% to 100% for Survey 2.

A significant interaction was evident for lesion nematode abundance/200 g soil between the two surveys, indicating that some of the fields had significantly different numbers of this genus (Annexure: Table A4.3b; Fig 4.3b). These localities were 4CS_2 (Free State province; Hoopstad area) and 10 MG (Free State province; Henneman area) with significantly higher lesion nematode abundance during 2020 compared to 2021 (indicated with green arrows in Fig. 4.3b) No significant differences in lesion nematode abundance were evident between the surveys for the other 16 fields.

Table 4.3a: Frequency of occurrence (FO), mean population density (MPD) and prominence values (PV) of *Pratylenchus* per 200 g soil samples of grain crops sampled from 18 fields situated in the Gauteng, North-West and Free State provinces of South Africa, during the 2020 (Survey 1) and 2021 (Survey 2) summer growing seasons.

Field no	Field code	Province	Crop sequence 2020/2021	2020 (Survey 1)			2021 (Survey 2)		
				FO	MPD	PV	FO	MPD	PV
1	1SH	Gauteng	Wheat/Soybean	0	0	0	50	4	3
2	2DM	North West	Sunflower/Maize	33	38	22	67	8	7
3	4CS_1	Free State	Maize/Maize	100	1	1	50	6	5
4	4CS_2	Free State	Maize/Maize	100	60	60	50	7	5
5	5JM	Free State	Maize/Maize	50	7	5	50	5	3
6	7WM	Free State	Sunflower/maize	100	69	69	100	35	35
7	8JB	Free State	Wheat/Soybean	33	255	147	67	55	45
8	10MG	Free State	Maize/Maize	100	380	380	83	26	23
9	11IM	Free State	Sunflower/Sunflower	67	13	10	33	7	4
10	13PvZ	Free State	Maize/Maize	100	18	18	83	16	14
11	14DV	Free State	Maize/Maize	100	27	27	100	8	8
12	16SJ	Free State	Maize/Maize	67	47	38	83	30	27
13	17JB_1	Free State	Maize/Soybean	33	17	10	67	49	40
14	17JB_2	Free State	Soybean/Maize	67	74	60	17	7	3
15	17JB_3	Free State	Wheat/Maize	100	49	49	100	16	16
16	18JF	Free State	Maize/Maize	67	73	60	50	20	14
17	20NR	North West	Sunflower/Maize	33	38	22	83	25	23
18	22PZ	Free State	Maize/soybean	100	79	79	100	548	548

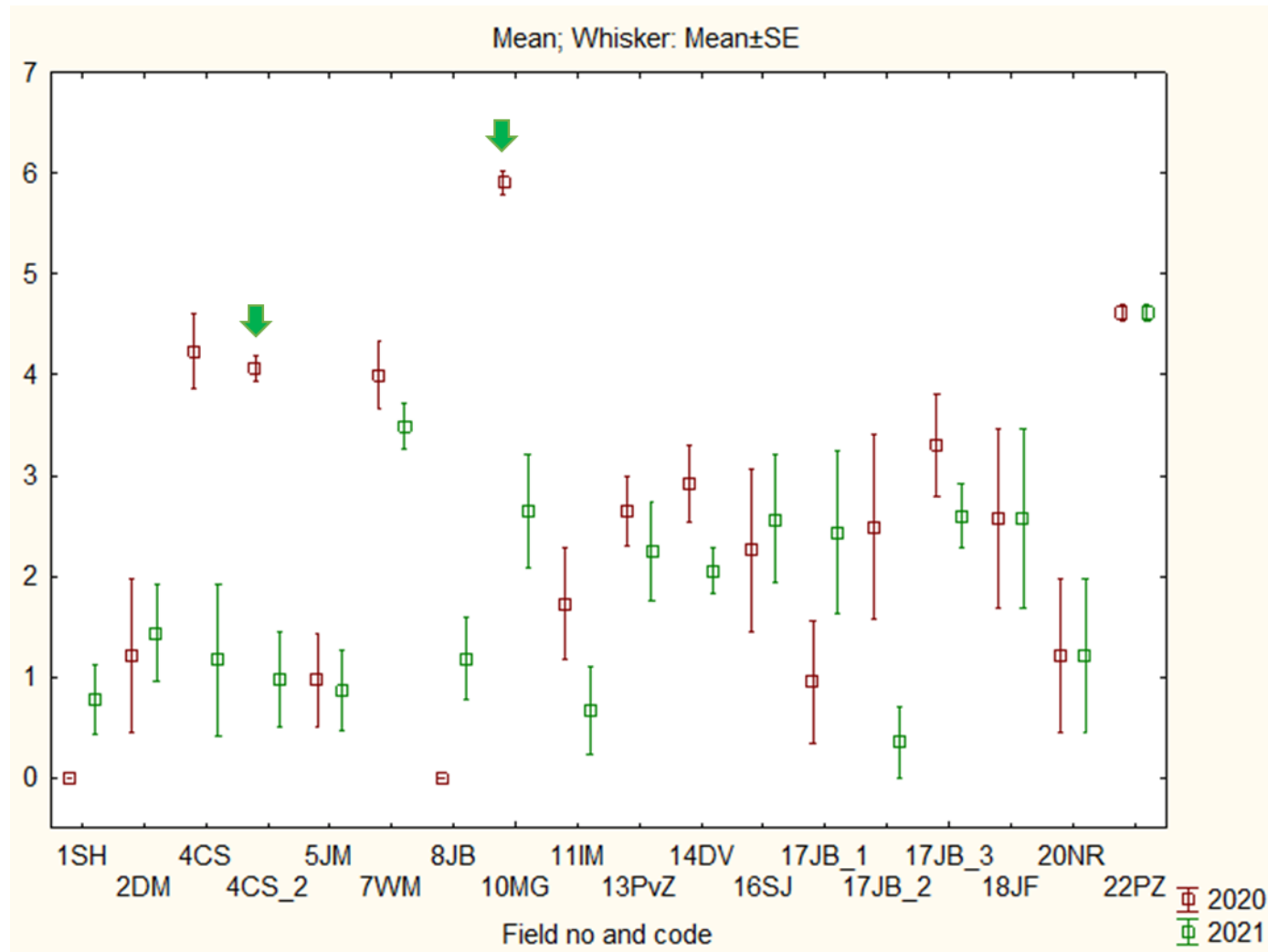


Figure 4.3a: *Pratylenchus* numbers/200 g soil sampled at farms of 18 grain producers during two consecutive growing seasons (2020 and 2021). The green arrows show significant differences in root-knot nematode numbers during the two growing seasons for those localities.

Dolichodoridae individuals were present in soil samples obtained from 4 and 14 fields for Surveys 1 and 2 respectively (Table 4.3a). The PV for Surveys 1 and 2 ranged from zero to 43 and from zero to 266, respectively. The ranges for MPD were from zero to 83 for Survey 1 and from zero to 266 for Survey 2, while the %FO ranged from zero to 75% and from zero to 100% for the respective surveys.

A significant interaction was evident for Dolichodoridae abundance/200 g soil between the two surveys, indicating that some of the fields had significantly different numbers of this genus (Annexure: Table A4.3a; Fig 4.3a). These localities were 1SH (Gauteng province; Carletonville area), 2DM (North West province; Lichtenburg area), 14DV (Free State province; Bothaville area), 17JB_3 (Free State province; Bothaville area) and 20NR (North West province; Ottosdal area) with significantly higher Dolichodoridae abundance during 2021 compared to 2020 (indicated with green arrows in Fig. 4.3b). No significant differences in Dolichodoridae abundance were evident for the other 13 fields between the two growing seasons.

Table 4.3b: Frequency of occurrence (FO), mean population density (MPD) and prominence values (PV) of Dolichodoridae per 200 g soil of grain crops sampled from 18 fields situated in the Gauteng, North-West and Free State provinces of South Africa, during the 2020 (Survey 1) and 2021 (Survey 2) summer growing seasons.

Field no	Field code	Province	Crop sequence 2020/2021	2020			2021		
				FO	MPD	PV	FO	MPD	PV
1	1SH	Gauteng	Wheat/Soybean	0	0	0	100	266	266
2	2DM	North West	Sunflower/Maize	17	83	34	83	116	106
3	4CS_1	Free State	Maize/Maize	75	50	43	50	3	2
4	4CS_2	Free State	Maize/Maize	0	0	0	17	26	10
5	5JM	Free State	Maize/Maize	0	0	0	17	4	1
6	7WM	Free State	Sunflower/maize	0	0	0	33	7	4
7	8JB	Free State	Wheat/Soybean	0	0	0	0	0	0
8	10MG	Free State	Maize/Maize	0	0	0	33	4	2
9	11IM	Free State	Sunflower/Sunflower	0	0	0	33	4	2
10	13PvZ	Free State	Maize/Maize	17	8	3	0	0	0
11	14DV	Free State	Maize/Maize	0	0	0	67	90	73
12	16SJ	Free State	Maize/Maize	0	0	0	0	0	0
13	17JB_1	Free State	Maize/Soybean	0	0	0	0	0	0
14	17JB_2	Free State	Soybean/Maize	0	0	0	67	8	7
15	17JB_3	Free State	Wheat/Maize	0	0	0	83	29	26
16	18JF	Free State	Maize/Maize	0	0	0	50	4	3
17	20NR	North West	Sunflower/Maize	17	83	34	100	200	200
18	22PZ	Free State	Maize/soybean	0	0	0	33	6	3

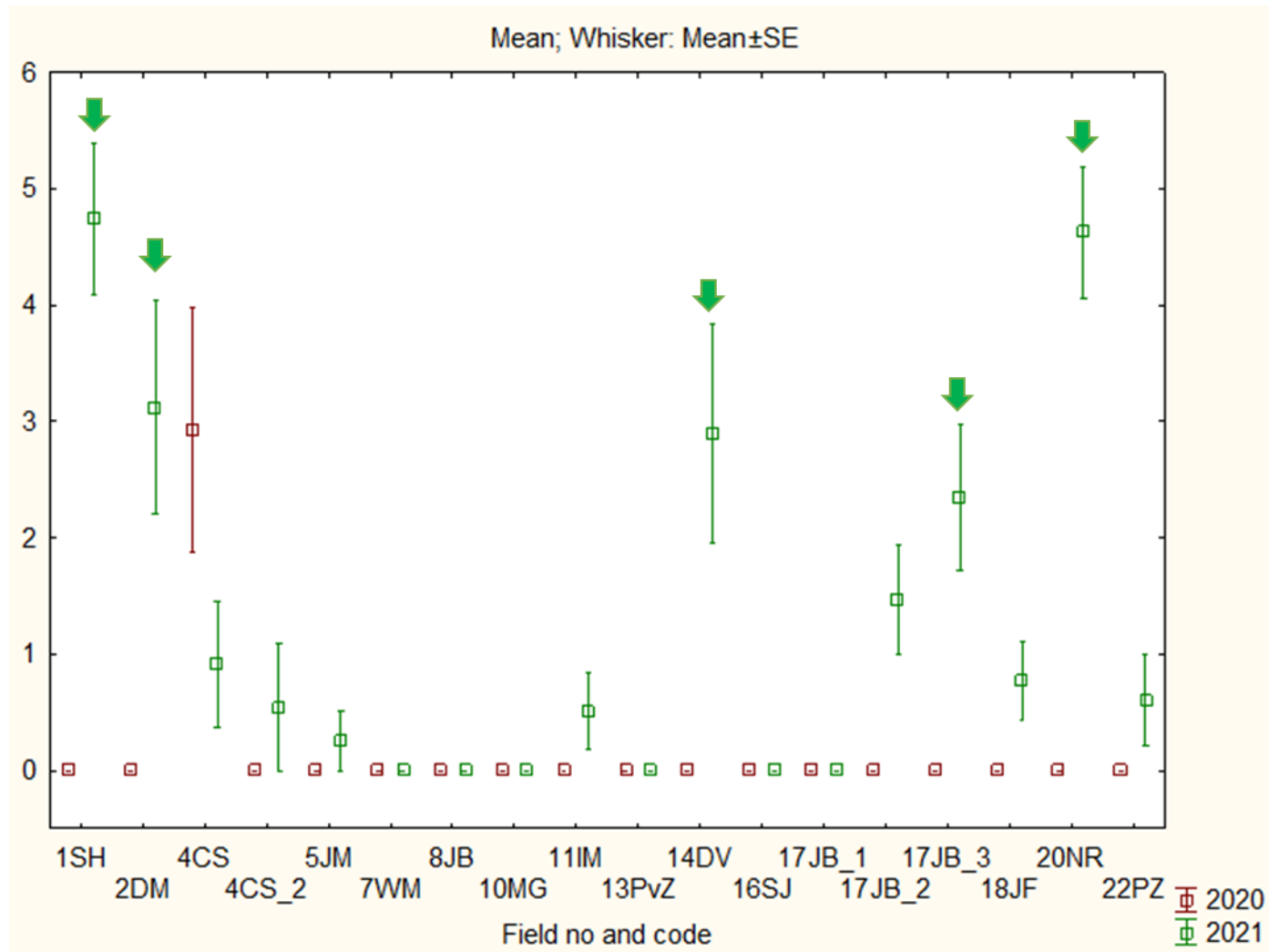


Figure 4.3b: Dolichodoridae numbers/20 g soil sampled at farms of 18 grain producers during two consecutive growing seasons (2020 and 2021). The green arrows show significant differences in root-knot nematode numbers during the two growing seasons for those localities.

Criconematidae individuals were present in soil samples obtained from 13 and 15 fields during Surveys 1 and 2, respectively (Table 4.3c). The PV values ranged from zero to 237 and from zero to 316 for the respective surveys and the corresponding MPD from zero to 237 and from zero to 346. The %FO ranged from zero to 100% for both surveys.

Hoplolaimidae individuals belonging to genera other than *Rotylenchulus* were present in soil samples obtained from 13 and 15 fields during Surveys 1 and 2, respectively (Table 4.3d). The PV and MPD values for this family both ranged from zero to 260 for Survey 1 and from zero to 179 for Survey 2. The %FO ranged from zero to 100% for both surveys.

Meloidogyne J2's were present in soil samples obtained from 11 and 14 fields during surveys 1 and 2, respectively (Table 4.3e). The PV for this genus ranged from zero to 172 and from zero to 27 for the two respective surveys, and the MPD from zero to 211 and from zero to 27. The %FO ranged from zero to 100% for both surveys.

Trichodoridae individuals were present in low population densities and only at 1 field during Survey 1, while for Survey 2 it occurred at 17 fields (Table 4.3f). The PV and MPD for this family both ranged from zero to 1 and from zero to 104 for Survey 1 and from zero to 104 for survey 2. The %FO ranged from zero to 100% for both surveys.

The PV for *Rotylenchulus* present in soil samples was obtained from 4 and 12 fields during Surveys 1 and 2, respectively (Table 4.3g). The PV for this genus ranged from zero to 51 and zero to 69 for the respective surveys, and the MPD from zero to 51 and from zero to 76. The %FO ranged from zero to 100% for both surveys.

Longidoridae individuals occurred in low population densities during both surveys and were present in soil samples obtained from 7 and 5 fields, respectively, during Surveys 1 and 2 (Table 4.3h). The PV values ranged from zero to 34 and from zero to 4 for the respective surveys, and the MPD from zero to 34 and from zero to 7. The %FO ranged from zero to 100% for Survey 1 and from zero to 33% for Survey 2.

Ditylenchus individuals occurred only during survey 2 and only in three fields (Table 4.3i). The PV values for this genus ranged from zero to 6, MPD from zero to 9, and the %FO from zero to 50%.

Table 4.3c: Frequency of occurrence (FO), mean population density (MPD) and prominence values (PV) of Criconematidae per 200 g soil samples of grain crops sampled from 18 fields situated in the Gauteng, North-West and Free State provinces of South Africa, during the 2020 (Survey 1) and 2021 (Survey 2) summer growing seasons.

Field no	Field code	Province	Crop sequence 2020/2021	2020 (Survey 1)			2021 (Survey 2)		
				FO	MPD	PV	FO	MPD	PV
1	1SH	Gauteng	Wheat/Soybean	0	0	0	0	0	0
2	2DM	North West	Sunflower/Maize	0	0	0	33	127	73
3	4CS_1	Free State	Maize/Maize	100	1	1	100	1	1
4	4CS_2	Free State	Maize/Maize	100	237	237	100	54	54
5	5JM	Free State	Maize/Maize	67	35	28	100	50	50
6	7WM	Free State	Sunflower/maize	100	35	35	0	0	0
7	8JB	Free State	Wheat/Soybean	83	12	11	0	0	0
8	10MG	Free State	Maize/Maize	0	0	0	83	29	26
9	11IM	Free State	Sunflower/Sunflower	67	16	13	50	5	3
10	13PvZ	Free State	Maize/Maize	100	63	63	83	46	42
11	14DV	Free State	Maize/Maize	83	139	127	83	346	316
12	16SJ	Free State	Maize/Maize	33	6	3	50	176	124
13	17JB_1	Free State	Maize/Soybean	17	2	1	67	53	43
14	17JB_2	Free State	Soybean/Maize	67	10	8	67	55	45
15	17JB_3	Free State	Wheat/Maize	33	23	13	100	31	31
16	18JF	Free State	Maize/Maize	100	107	107	100	62	62
17	20NR	North West	Sunflower/Maize	0	0	0	0	0	0
18	22PZ	Free State	Maize/soybean	0	0	0	17	4	1

Table 4.3d: Frequency of occurrence (FO), mean population density (MPD) and prominence values (PV) of Hoplolaimidae per 200 g soil samples of grain crops sampled from 18 fields situated in the Gauteng, North-West and Free State provinces of South Africa, during the 2020 (Survey 1) and 2021 (Survey 2) summer growing seasons.

Field no	Field code	Province	Crop sequence 2020/2021	2020 (Survey 1)			2021 (Survey 2)		
				FO	MPD	PV	FO	MPD	PV
1	1SH	Gauteng	Wheat/Soybean	100	52	52	83	22	20
2	2DM	North West	Sunflower/Maize	100	260	260	67	54	44
3	4CS_1	Free State	Maize/Maize	0	0	0	0	0	0
4	4CS_2	Free State	Maize/Maize	50	10	7	0	0	0
5	5JM	Free State	Maize/Maize	0	0	0	67	6	5
6	7WM	Free State	Sunflower/maize	83	37	34	67	6	5
7	8JB	Free State	Wheat/Soybean	0	0	0	0	0	0
8	10MG	Free State	Maize/Maize	50	49	35	67	16	13
9	11IM	Free State	Sunflower/Sunflower	0	0	0	33	26	15
10	13PvZ	Free State	Maize/Maize	83	13	12	100	42	42
11	14DV	Free State	Maize/Maize	100	55	55	50	17	12
12	16SJ	Free State	Maize/Maize	67	28	23	50	86	60
13	17JB_1	Free State	Maize/Soybean	17	4	2	67	10	8
14	17JB_2	Free State	Soybean/Maize	33	23	13	50	4	3
15	17JB_3	Free State	Wheat/Maize	67	74	60	33	17	10
16	18JF	Free State	Maize/Maize	0	0	0	33	9	5
17	20NR	North West	Sunflower/Maize	100	260	260	100	179	179
18	22PZ	Free State	Maize/soybean	100	200	200	100	139	139

Table 4.3e: Frequency of occurrence (FO), mean population density (MPD) and prominence values (PV) *Meloidogyne* per 200 g soil samples of grain crops sampled from 18 fields situated in the Gauteng, North-West and Free State provinces of South Africa, during the 2020 (Survey 1) and 2021 (Survey 2) summer growing seasons.

Field no	Field code	Province	Crop sequence 2020/2021	2020 (Survey 1)			2021 (Survey 2)		
				FO	MPD	PV	FO	MPD	PV
1	1SH	Gauteng	Wheat/Soybean	0	0	0	83	27	27
2	2DM	North West	Sunflower/Maize	0	0	0	100	26	26
3	4CS_1	Free State	Maize/Maize	50	29	20	25	4	2
4	4CS_2	Free State	Maize/Maize	17	13	5	0	0	0
5	5JM	Free State	Maize/Maize	67	5	4	33	18	11
6	7WM	Free State	Sunflower/maize	0	0	0	17	4	1
7	8JB	Free State	Wheat/Soybean	17	50	20	0	0	0
8	10MG	Free State	Maize/Maize	0	0	0	0	0	0
9	11IM	Free State	Sunflower/Sunflower	100	118	118	17	7	3
10	13PvZ	Free State	Maize/Maize	100	40	40	33	11	6
11	14DV	Free State	Maize/Maize	83	110	100	50	17	12
12	16SJ	Free State	Maize/Maize	50	7	5	50	23	16
13	17JB_1	Free State	Maize/Soybean	100	13	13	17	4	1
14	17JB_2	Free State	Soybean/Maize	100	49	49	17	4	1
15	17JB_3	Free State	Wheat/Maize	0	0	0	33	7	4
16	18JF	Free State	Maize/Maize	67	211	172	0	0	0
17	20NR	North West	Sunflower/Maize	0	0	0	17	4	1
18	22PZ	Free State	Maize/soybean	0	0	0	17	4	1

Table 4.3f: Frequency of occurrence (FO), mean population density (MPD) and prominence values (PV) of Trichodoridae per 200 g soil of grain crops sampled from 18 fields situated in the Gauteng, North-West and Free State provinces of South Africa, during the 2020 (Survey 1) and 2021 (Survey 2) summer growing seasons.

Field no	Field code	Province	Crop sequence 2020/2021	2020 (Survey 1)			2021 (Survey 2)		
				FO	MPD	PV	FO	MPD	PV
1	1SH	Gauteng	Wheat/Soybean	0	0	0	33	4	2
2	2DM	North West	Sunflower/Maize	0	0	0	100	88	88
3	4CS_1	Free State	Maize/Maize	0	0	0	75	10	9
4	4CS_2	Free State	Maize/Maize	0	0	0	0	0	0
5	5JM	Free State	Maize/Maize	0	0	0	100	29	29
6	7WM	Free State	Sunflower/maize	0	0	0	100	104	104
7	8JB	Free State	Wheat/Soybean	100	1	1	100	26	26
8	10MG	Free State	Maize/Maize	0	0	0	100	28	28
9	11IM	Free State	Sunflower/Sunflower	0	0	0	100	20	20
10	13PvZ	Free State	Maize/Maize	0	0	0	100	54	54
11	14DV	Free State	Maize/Maize	0	0	0	100	20	20
12	16SJ	Free State	Maize/Maize	0	0	0	100	33	33
13	17JB_1	Free State	Maize/Soybean	0	0	0	83	41	37
14	17JB_2	Free State	Soybean/Maize	0	0	0	83	54	49
15	17JB_3	Free State	Wheat/Maize	0	0	0	100	48	48
16	18JF	Free State	Maize/Maize	0	0	0	100	26	26
17	20NR	North West	Sunflower/Maize	0	0	0	50	29	21
18	22PZ	Free State	Maize/soybean	0	0	0	67	8	7

Table 4.3g: Frequency of occurrence (FO), mean population density (MPD) and prominence values (PV) of *Rotylenchulus* per 200 g soil samples of grain crops sampled from 18 fields situated in the Gauteng, North-West and Free State provinces of South Africa, during the 2020 (Survey 1) and 2021 (Survey 2) summer growing seasons.

Field no	Field code	Province	Crop sequence 2020/2021	2020 (Survey 1)			2021 (Survey 2)		
				FO	MPD	PV	FO	MPD	PV
1	1SH	Gauteng	Wheat/Soybean	33	9	5	0	0	0
2	2DM	North West	Sunflower/Maize	0	0	0	83	76	69
3	4CS_1	Free State	Maize/Maize	0	0	0	0	0	0
4	4CS_2	Free State	Maize/Maize	0	0	0	0	0	0
5	5JM	Free State	Maize/Maize	0	0	0	0	0	0
6	7WM	Free State	Sunflower/maize	17	44	18	33	4	2
7	8JB	Free State	Wheat/Soybean	17	6	2	0	0	0
8	10MG	Free State	Maize/Maize	0	0	0	100	13	13
9	11IM	Free State	Sunflower/Sunflower	0	0	0	0	0	0
10	13PvZ	Free State	Maize/Maize	0	0	0	83	13	12
11	14DV	Free State	Maize/Maize	0	0	0	33	7	4
12	16SJ	Free State	Maize/Maize	0	0	0	67	8	7
13	17JB_1	Free State	Maize/Soybean	0	0	0	33	4	2
14	17JB_2	Free State	Soybean/Maize	0	0	0	50	4	3
15	17JB_3	Free State	Wheat/Maize	0	0	0	33	6	3
16	18JF	Free State	Maize/Maize	0	0	0	67	9	7
17	20NR	North West	Sunflower/Maize	0	0	0	100	42	42
18	22PZ	Free State	Maize/soybean	100	51	51	100	33	33

Table 4.3h: Frequency of occurrence (FO), mean population density (MPD) and prominence values (PV) of Longidoridae per 200 g soil of grain crops sampled from 18 fields situated in the Gauteng, North-West and Free State provinces of South Africa, during the 2020 (Survey 1) and 2021 (Survey 2) summer growing seasons.

Field no	Field code	Province	Crop sequence 2020/2021	2020			2021		
				FO	MPD	PV	FO	MPD	PV
1	1SH	Gauteng	Wheat/Soybean	0	0	0	0	0	0
2	2DM	North West	Sunflower/Maize	0	0	0	33	2	1
3	4CS_1	Free State	Maize/Maize	0	0	0	0	0	0
4	4CS_2	Free State	Maize/Maize	0	0	0	0	0	0
5	5JM	Free State	Maize/Maize	33	2	1	0	0	0
6	7WM	Free State	Sunflower/maize	0	0	0	17	7	3
7	8JB	Free State	Wheat/Soybean	17	0	0	0	0	0
8	10MG	Free State	Maize/Maize	0	0	0	0	0	0
9	11IM	Free State	Sunflower/Sunflower	0	0	0	0	0	0
10	13PvZ	Free State	Maize/Maize	17	2	1	33	7	4
11	14DV	Free State	Maize/Maize	0	0	0	0	0	0
12	16SJ	Free State	Maize/Maize	50	4	3	0	0	0
13	17JB_1	Free State	Maize/Soybean	50	10	7	0	0	0
14	17JB_2	Free State	Soybean/Maize	100	34	34	0	0	0
15	17JB_3	Free State	Wheat/Maize	67	10	8	17	4	1
16	18JF	Free State	Maize/Maize	0	0	0	0	0	0
17	20NR	North West	Sunflower/Maize	0	0	0	0	0	0
18	22PZ	Free State	Maize/soybean	0	0	0	33	7	4

Table 4.3i: Frequency of occurrence (FO), mean population density (MPD) and prominence values (PV) of *Ditylenchus* per 200 g soil samples of grain crops sampled from 18 fields situated in the Gauteng, North-West and Free State provinces of South Africa, during the 2020 (Survey 1) and 2021 (Survey 2) summer growing seasons.

Field no	Field code	Province	Crop sequence 2020/2021	2020 (Survey 1)			2021 (Survey 2)		
				FO	MPD	PV	FO	MPD	PV
1	1SH	Gauteng	Wheat/Soybean	0	0	0	0	0	0
2	2DM	North West	Sunflower/Maize	0	0	0	0	0	0
3	4CS_1	Free State	Maize/Maize	0	0	0	0	0	0
4	4CS_2	Free State	Maize/Maize	0	0	0	17	4	2
5	5JM	Free State	Maize/Maize	0	0	0	0	0	0
6	7WM	Free State	Sunflower/maize	0	0	0	0	0	0
7	8JB	Free State	Wheat/Soybean	0	0	0	0	0	0
8	10MG	Free State	Maize/Maize	0	0	0	0	0	0
9	11IM	Free State	Sunflower/Sunflower	0	0	0	0	0	0
10	13PvZ	Free State	Maize/Maize	0	0	0	50	9	6
11	14DV	Free State	Maize/Maize	0	0	0	0	0	0
12	16SJ	Free State	Maize/Maize	0	0	0	0	0	0
13	17JB_1	Free State	Maize/Soybean	0	0	0	0	0	0
14	17JB_2	Free State	Soybean/Maize	0	0	0	0	0	0
15	17JB_3	Free State	Wheat/Maize	0	0	0	17	4	1
16	18JF	Free State	Maize/Maize	0	0	0	0	0	0
17	20NR	North West	Sunflower/Maize	0	0	0	0	0	0
18	22PZ	Free State	Maize/soybean	0	0	0	0	0	0

4.2.3.2 Beneficial nematodes

During Survey 1 the beneficial nematodes were counted only as one group and indicated as beneficial nematodes due to logistic challenges experienced (Table 4.4a). The families and trophic groups (bacterivores, fungivores, omnivores and predators) the beneficial nematodes belong to were, however, distinguished during Survey 2.

Beneficial nematodes were present in 200 g soil samples obtained from 16 and 18 fields during Surveys 1 and 2, respectively. The PV and MPD values both ranged from zero to 343 for Survey 1 and from 72 to 953 for Survey 2. The %FO was 88.9% for Survey 1 and was 100% for Survey 2.

During Survey 2, bacterivores dominated followed by fungivores, predators and omnivores (Table 4.4b). The 22 families of beneficial nematodes, with their nematode-based index values (NBIs), identified from the 18 fields sampled during Survey 2 are listed in Table 4.4c.

Both the PV and MPD values for bacterivores ranged from 47 to 797, while the %FO was 100% (Table 4.4b). For fungivores, the PV and MPD values ranged from 1 to 120, with the %FO being 100%. Both the PV and MPD values for omnivores ranged from 1 to 39, while for predators these respective values ranged from zero to 20. For omnivores, the %FO ranged from 17 to 100% and for predators from zero to 100%.

Table 4.4a: Frequency of occurrence (FO), mean population density (MPD) and prominence values (PV) of beneficial nematodes (representing bacterivores, fungivores, omnivores and predators) per 200 g soil of grain crops sampled from 18 fields situated in the Gauteng, North-West and Free State provinces of South Africa, during 2020 (Survey 1) and 2021 (Survey 2) summer growing seasons.

Field no	Field code	Province	Crop sequence 2020/2021	2020 (Survey 1)			2021 (Survey 2)		
				FO	MPD	PV	FO	MPD	PV
1	1SH	Gauteng	Wheat/Soybean	100	343	343	100	639	639
2	2DM	North West	Sunflower/Maize	100	36	36	100	352	352
3	4CS_1	Free State	Maize/Maize	100	1	1	100	110	110
4	4CS_2	Free State	Maize/Maize	100	10	10	100	241	241
5	5JM	Free State	Maize/Maize	100	30	30	100	191	191
6	7WM	Free State	Sunflower/maize	100	35	35	100	368	368
7	8JB	Free State	Wheat/Soybean	100	316	316	100	209	209
8	10MG	Free State	Maize/Maize	100	47	47	100	283	283
9	11IM	Free State	Sunflower/Sunflower	100	30	30	100	72	72
10	13PvZ	Free State	Maize/Maize	100	21	21	100	254	254
11	14DV	Free State	Maize/Maize	100	25	25	100	183	183
12	16SJ	Free State	Maize/Maize	100	18	18	100	131	131
13	17JB_1	Free State	Maize/Soybean	0	0	0	100	275	275
14	17JB_2	Free State	Soybean/Maize	0	0	0	100	79	79
15	17JB_3	Free State	Wheat/Maize	100	34	34	100	147	147
16	18JF	Free State	Maize/Maize	100	48	48	100	85	85
17	20NR	North West	Sunflower/Maize	100	36	36	100	623	623
18	22PZ	Free State	Maize/soybean	100	148	148	100	953	953

Table 4.4b: Frequency of occurrence (FO), mean population density (MPD) and prominence values (PV) of bacterivores, fungivores, omnivores and predators per 200 g soil of grain crops sampled from 18 fields situated in the Gauteng, North-West and Free State provinces of South Africa, during the 2021 (Survey 2) summer growing seasons.

Field no	Field code	Province	Bacterivores			Fungivores			Omnivores			Predators		
			FO	MPD	PV	FO	MPD	PV	FO	MPD	PV	FO	MPD	PV
1	1SH	Gauteng	100	298	298	100	43	43	17	1	1	50	9	9
2	2DM	North West	100	184	184	100	41	41	83	6	6	100	17	17
3	4CS_1	Free State	100	126	126	100	28	28	25	3	3	0	0	0
4	4CS_2	Free State	100	204	204	100	27	27	17	3	3	33	3	3
5	5JM	Free State	100	143	143	100	25	25	100	9	9	50	4	4
6	7WM	Free State	100	189	189	100	120	120	67	5	5	67	15	15
7	8JB	Free State	100	54	54	100	37	37	100	10	10	50	2	2
8	10MG	Free State	100	171	171	100	83	83	67	10	10	50	2	2
9	11IM	Free State	100	47	47	67	9	9	50	6	6	67	6	6
10	13PvZ	Free State	100	190	190	100	26	26	83	4	4	100	15	15
11	14DV	Free State	100	67	67	100	31	31	50	5	5	67	13	13
12	16SJ	Free State	100	78	78	83	32	32	33	2	2	83	10	10
13	17JB_1	Free State	100	209	209	100	28	28	83	8	8	83	17	17
14	17JB_2	Free State	100	56	56	33	1	1	33	1	1	67	11	11
15	17JB_3	Free State	100	76	76	100	13	13	67	9	9	100	14	14
16	18JF	Free State	100	70	70	100	5	5	17	1	1	83	4	4
17	20NR	North West	100	257	257	100	107	107	50	3	3	100	9	9
18	22PZ	Free State	100	797	797	83	70	70	67	39	39	100	20	20

Table 4.4c: The genera and families of beneficial nematodes identified per 200 g soil of grain crops sampled from 18 fields situated in the Gauteng, North-West and Free State provinces of South Africa, during the 2021 (Survey 2) summer growing season.

Family	c-p class	Trophic group
Alaimidae	4	Bacterivores
Cephalobidae	2	Bacterivores
Monhysteridae	2	Bacterivores
Panagrolaimidae	1	Bacterivores
Plectidae	2	Bacterivores
Rhabditidae	1	Bacterivores
Aphelenchidae	2	Fungivores
Aphelenchoides	2	Fungivores
Diphtherophoridae	3	Fungivores
Iotonchiidae	4	Fungivores
Leptonchidae	4	Fungivores
Neotylenchidae	2	Fungivores
Dorylaimidae	4	Omnivores
Nordiidae	4	Omnivores
Qudsianematidae	4	Omnivores
Thornenematidae	5	Omnivores
Actinolaimidae	5	Predators
Aporcelaimidae	5	Predators
Discolaimoides	4	Predators
Mononchidae	4	Predators
Nygolaimidae	5	Predators
Tripylidae	3	Predators

4.2.3.2.1 The role of beneficial nematodes in the food web

a) Faunal analysis

According to the soil faunal analysis (Fig. 4.4), the majority of the fields sampled (83%) grouped in Quadrat D representing stress and depleted soils. These fields had low EI values ranging from 11.65 (field 4) to 47.77 (field 1) and also low SI that ranged from 7.86 (field 3) to 44.41 (field 9) (Table 4.4d).

Eleven percent of the fields plotted in Quadrat A which represent enriched but unstructured nematode communities. These 2 fields (5 and 16) had intermediate EI values and low SI values (Table 4.4). Only one field (15) plotted in Quadrat C (matured and fertile) showing an intermediate to high SI of 61.69, but a low EI of 28.29. None of the fields plotted in Quadrat B (representing a stable and enriched soil ecosystem).

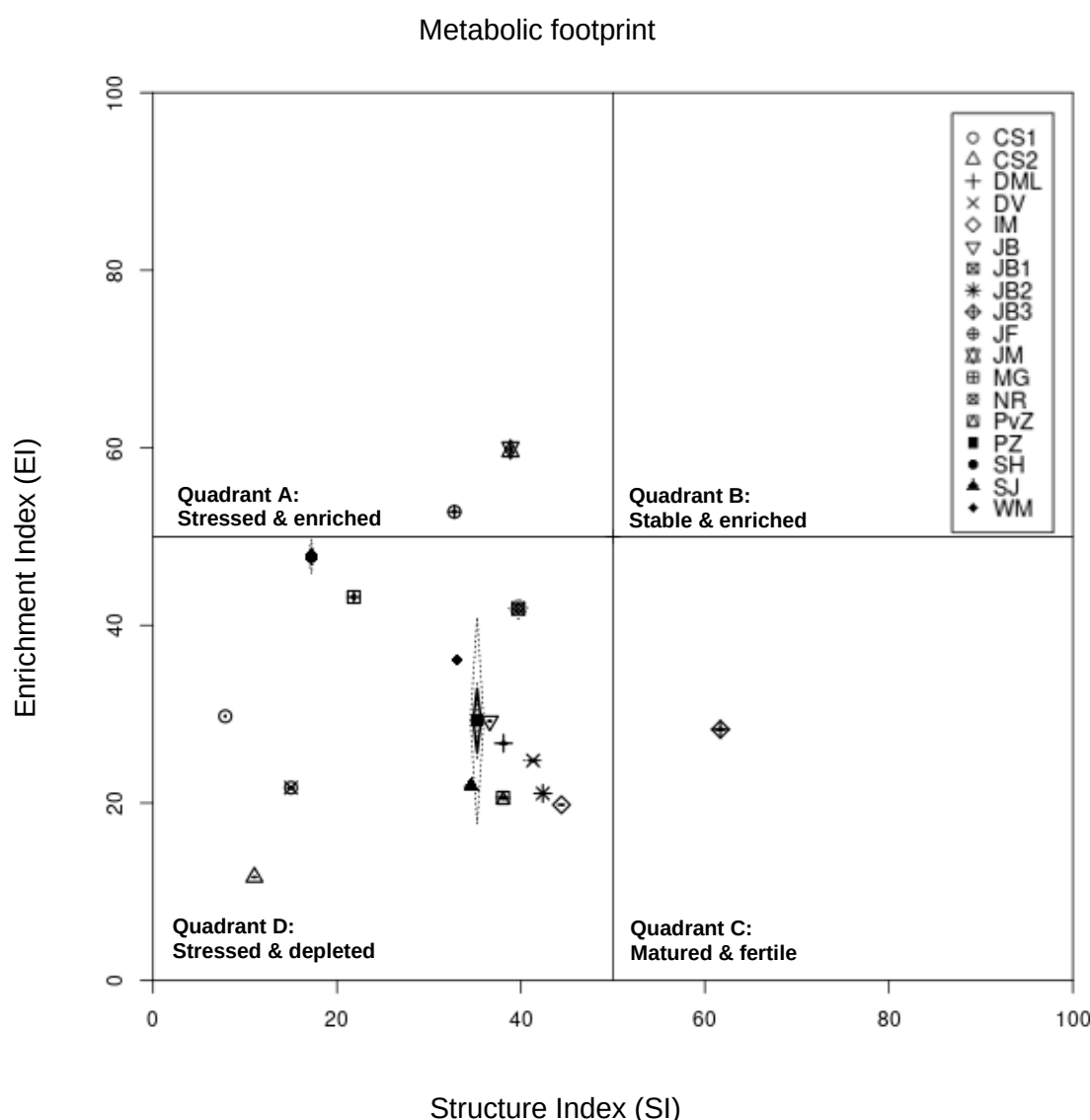


Figure 4.4: A faunal analysis illustrating the metabolic footprint, according to Structure (SI) and Enrichment (EI) indices, of beneficial nematode assemblages occurring at each of the 18 fields sampled during the 2021 nematode survey as identified from 200 g soil samples.

b) Nematode-based indices (NBIs)

In this study significant differences ($P < 0.05$) were evident for the MI 2-5 across the 18 fields sampled with fields 11 (14DV) and 14 (17JB_2) having the highest values which differed significantly from fields 1 (1SH), 3 (4CS) and 4 (4CS_2) with the lowest values (Table 4.4d). Fields 11 and 14 however, did not differ significantly from the other fields in terms of their MI 2-5 values.

Concerning the SI, fields 4 and 17 (20NR) had the lowest values which differed significantly from the following fields with the highest values (in descending order): 15 (17JB_3), 9

(11IM), 14 (17JB_2), 13 (17JB_1) and 11 (14DV) (Table 4.4d). Field 15 did however not differ significantly from fields 5, 9, 10, 11, 13 and 14 but did differ significantly from fields 1, 3, 4, 6, 8, 12, 16, 17 and 18.

For EI, the fields also differed significantly with field 5 (5JM) having the highest value, which was similar to those of fields 1, 8 and 16 but differed significantly from the fields (Table 4.4d).

Significant differences were evident for the CI too with fields 4 and 11 having the highest values of 100 which differed significantly from all the other fields except from fields 7, 12 and 17 (Table 4.4d).

Table 4.4d: Mean and standard deviation (SD) of nematode-based index values (NBIs) (MI 2-5 = Maturity index 2-5, EI = Enrichment index, SI = Structure index, CI = Channel index) for 18 fields situated in the Gauteng, North-West and Free State provinces of South Africa, during the 2021 (Survey 2) summer growing seasons.

Field no	Field code	Province	Crop sequence 2020/2021	NBIs			
				MI 2-5	SI	EI	CI
1	1SH	Gauteng	Wheat/Soybean	2.12 (±0.05) ab	17.21 (±7.84) abc	47.77 (±8.21) defg	18.37(±8.21) abc
2	2DM	North West	Sunflower/Maize	2.28(±0.04) abcd	38.11(±3.72) bcde	26.72(±2.79) abc	50.66(±12.43) def
3	4CS	Free State	Maize/Maize	2.05(±0.05) ab	7.86(±7.86) a	29.76(±10.62) abcd	64.03(±13.54) efgh
4	4CS_2	Free State	Maize/Maize	2.08(±0.06) a	11.02(±8.10) a	11.65(±3.13) a	100.00(±0.00) j
5	5JM	Free State	Maize/Maize	2.29(±0.04) abcd	38.86(±4.17) bcde	59.82(±4.29) g	12.84(±3.76) ab
6	7WM	Free State	Sunflower/Maize	2.27(±0.10) abcd	33.09(9.84) abcd	36.08(4.28) bcdef	70.41(±8.54) fghi
7	8JB	Free State	Wheat/Soybean	2.26(±0.03) abcd	36.61(±2.78) bcd	29.22(±5.46) bc	94.09(±5.91) hij
8	10MG	Free State	Maize/Maize	2.14(±0.04) abc	21.83(±5.85) abcd	43.19(±4.07) cdefg	43.85(±7.21) cdef
9	11IM	Free State	Sunflower/Sunflower	2.40(±0.10) bcde	44.41(±9.76) de	19.79(±4.88) ab	64.00(±20.33) efg
10	13PvZ	Free State	Maize/Maize	2.29(±0.07) abcd	38.06(±6.51) bcde	20.59(±2.19) ab	49.85(±11.64) def
11	14DV	Free State	Maize/Maize	2.44(±0.19) cde	41.32(±12.48) cde	24.78(±1.54) ab	100.00(±0.00) j
12	16SJ	Free State	Maize/Maize	2.31(±0.13) abcd	34.58(±10.49) abcd	21.92(±6.11) ab	92.00(±7.30) ghij
13	17JB_1	Free State	Maize/Soybean	2.34(±0.11) abcde	39.72(±9.53) cde	41.89(±4.16) cdef	27.29(±14.87) abcd
14	17JB_2	Free State	Soybean/Maize	2.44(±0.15) cde	42.38(±13.58) de	21.07(±9.36) ab	27.78(±19.77) abcd
15	17JB_3	Free State	Wheat/Maize	2.63(±0.09) e	61.69(±4.65) e	28.29(±7.42) abc	64.50s(±19.87) efg
16	18JF	Free State	Maize/Maize	2.22(±0.04) abc	32.77(±0.12) abcd	52.79(±0.12) fg	8.09(±1.51) a
17	20NR	North West	Sunflower/Maize	2.09(±0.02) ab	15.00(±3.53) ab	21.71(±5.31) ab	95.66(±2.85) ij
18	22PZ	Free State	Maize/Soybean	2.13(±0.12) abcd	35.23(±9.87) abcd	29.27(±13.92) bcde	33.85(±12.87) bcde
P value				0.027*	0.009*	0.001*	0.000*
F ratio				1 890	2 160	4 940	10.12

4.3 Relationships between nematodes and selected abiotic and other parameters

No strong correlations were evident when PCA analysis was done for nematodes (beneficial and plant-parasitic) and selected abiotic parameters (rainfall, temperature, % clay) or other parameters (NBIs, cropping system, dry land and irrigated fields and nematicide applied) for either Survey 1 or 2 (see Annexure 1; Fig. 1 – Fig 4).

4.4 Discussion and conclusion

The top 3 plant-parasitic nematode genera present in root samples from local grain crops (maize, soybean, sunflower and wheat) sampled during two consecutive surveys (2020 and 2021) were represented by root-knot nematodes, spiral nematodes (belonging to the genus *Rotylenchulus*) and lesion nematodes. The former two genera are sessile endoparasites while lesion nematodes are migratory endoparasites (Moens and Perry, 2009). These results agree with data from a recent report for maize by Bekker *et al.* (2022) and are substantiated by data for maize and sunflower generated during 2019 and 2020 surveys that were conducted in fields of New-Era producers (Qwabe, 2023). For soil samples, the predominance of lesion nematodes in grain crop rhizosphere soils is not an unexpected finding but the listing of the ectoparasitic families Dolichodoridae and Criconeematidae in the second and third places, respectively, is novel and interesting since it was not reported before.

The presence of root-knot nematodes and *Rotylenchulus* in roots of maize (De Waele and Jordaan 1988), soybean (Fourie *et al.*, 2001a), sunflower (Bolton *et al.*, 1989) and wheat (Jordaan *et al.*, 1992) has been documented in SA during the first surveys that were done for these crops. However, during these initial surveys, the PV of these two genera were relatively low for maize, sunflower and wheat compared to those recorded during this current survey and the one by Bekker *et al.* (2022). The high PV of root-knot nematodes in the roots of maize and soybean also agrees with earlier reports by Riekert and Henshaw (1998) and Riekert (1996) who recorded this genus as being predominant in maize-based grain rotations. Moreover, according to Pretorius (2018), root-knot nematodes dominated in maize roots during the 2013/14 survey too (highest PV of 38479 individuals/50 g roots), which was in agreement with this study. For soybean, a high PV of 953 individuals/50 g roots was recorded for root-knot nematodes during the first study in the early 2002s (Fourie *et al.*, 2001a) which is substantiated by the results of this current study. Furthermore, recording root-knot nematodes as the predominant genus infecting soybean (PV of 8915 individuals/50 g), also agree with the high PV reported by Engelbrecht *et al.* (2021) and Mbatyoti *et al.* (2021) (high PV of 5291 individuals/20 g roots and 236680 individuals/50 g roots respectively). In addition, this study also agrees with the survey by Qwabe (2023) for maize and sunflower crops sampled during the 2020 summer growing season (PV of 110344 and 38463 individuals/50 g roots, respectively).

Except for root-knot nematodes, lesion nematodes have been reported as another plant-parasitic nematode genus that widely occurs in high abundance infecting maize and soybean roots (Mbatyoti *et al.*, 2021; Pretorius, 2018; Bekker, 2008; Fourie *et al.*, 2001a; De Waele and Jordaan 1988). Although the highest PV of 366 individuals/5 g roots recorded for lesion nematodes in this study was lower than that recorded by Pretorius (2018) (PV of 899 individuals/5 g roots; for a no-tilled, rain-fed maize field, Sannieshof area, North West Province) it is still in a comparative range indicating the risk this genus poses in maize fields. Bekker *et al.* (2022) reported relatively low PV for lesion nematodes in maize, namely 53 individuals/5 g for conservation (CA) maize and 149 individuals/5 g for conventional maize (CTA). Maina *et al.* (2019), however, recently reported it as the predominant nematode genus infecting maize in Kenya. For soybean, the PV of 112 individuals/5 g roots for lesion nematodes reported by Fourie *et al.* (2001a) was considerably lower than the PV of 1198 individuals/5 g roots reported recently by Mbatyoti *et al.* (2021) but comparatively in the same range as that recorded during the current study and that reported by Engelbrecht *et al.* (2021) (highest PV of 402.8 individuals/20 g roots). Concerning sunflower, the highest PV of 18 individuals/5 g roots for two sunflower fields sampled (11IM, Hoopstad area, Free State and 20NR, Ottosdal area, North West) were in a similar range as findings by Bolton *et al.* (1989), namely 53 individuals/5 g roots. For wheat, De Waele and Jordaan (1988a) listed *Pratylenchus* as the dominant genus in the late 1980s with the highest PV of 832 individuals/5 g roots. In this current survey study only three wheat fields were sampled with lesion nematodes present in two of them, but with relatively low PV of 20 and 70 individuals/5 g roots, respectively). This data, however, comparatively corresponds well with data from Morocco for wheat where *Pratylenchus* dominated with a PV range of 67 to 100 individuals/10 g roots (Mokrini *et al.*, 2016; 2012). Moreover, Waceke *et al.* (2013) communicated that in both root and maize samples, *Pratylenchus* was the most predominant genus occurring in 100 and 96% of soil and maize root samples, respectively.

An interesting phenomenon emanating from the current survey study is the high abundance and wide occurrence of *Rotylenchulus* individuals in roots of maize and soybean crops; this is in agreement with recent reports by Bekker *et al.* (2022) and Mbatyoti *et al.* (2021). This trend was also substantiated by data from the diagnostic analyses at the NWU Nematology Laboratory (Personal communication, H. Fourie, January 2023). The PV ranges for *Rotylenchulus* in roots of soybean as reported by Engelbrecht *et al.* (2021), Mbatyoti *et al.* (2021) and Fourie *et al.* (2001a) of 113 individuals/20 g, 436 individuals/5 g and 1 individual/5 g, roots respectively, is comparatively lower than the high PV of 978/50 g roots recorded for this genus during this current survey. The latter high PV for this genus in roots of grain crops, however, corresponds with high PV values (2674/50 g) reported by Bekker *et al.* (2022) for maize under conservation agricultural practices. The New-Era grain producer survey by Qwabe (2023) showed exceptionally high PV for *Rotylenchulus*, namely 6368 and 2129 individuals/50 g roots of sunflower and maize crops, respectively. Interestingly, two fields 22PZ and 17JB_1 with high PV for *Rotylenchulus* of 978 and

447 individuals/50 g roots, respectively, suggest that maize rotated with soybean and/or sunflower favors high build-ups in population densities of this genus. The recent findings that this genus occurs in high abundance in local grain production areas are in contrast to first records that it commonly occurred in low abundance in grain crop fields, e.g., maize (De Waele and Jordaan, 1988; the PV 29 individuals/5 g roots), wheat (Jordaan *et al.*, 1992), groundnut (*Arachis hypogaea* L.; Steenkamp *et al.*, 2015; Venter *et al.*, 1992) and sunflower (Bolton *et al.*, 1989). It is hence agreed with Bekker *et al.* (2016) that the potential impact of this genus on grain crops is still unknown and that the use of the adapted NaOCl method (Riekert, 1995) and distinguishing between eggs of *Rotylenchulus* and *Meloidogyne* occurring together in samples (Bekker *et al.*, 2016) contributed to detect its presence. The reason for the increased *Rotylenchulus* abundance in local grain crop fields and whether it is an economically important pest of grain crops can, however, not be explained at this stage since further research is needed to investigate this phenomenon.

For soil samples, the domination of endoparasitic lesion nematodes, followed by the ectoparasitic families Dolichodoridae and Criconematidae, semi-endoparasitic Hoplolaimidae and endoparasitic root-knot nematodes resulted in novel and interesting data. Although results from this study agree with those by Pretorius (2018) for a recent maize survey in that *Pratylenchus* dominated in soil samples (Pretorius, 2018), it disagreed with other recent reports. For example, Bekker *et al.* (2022) and Mbatyoti *et al.* (2021) indicated that *Rotylenchulus* (PV of 434 and 2145 individuals/200 g soil, respectively) and *Scutellonema* (PV of 155 and 1217 individuals/200 g soil, respectively) dominated in soil samples of maize and soybean, respectively. Data from this current study also disagreed with data from the New-Era survey by Qwabe (2023) showing that *Rotylenchulus* (PV of 1381 individuals/200 g soil) and *Helicotylenchus* (PV of 2270 individuals/200 g soil) dominated. Cognisance should be taken about the high abundance and wide occurrence of Dolichodoridae and Criconematidae individuals in local grain production fields. Producers should be proactive in aiming to suppress population densities of such plant-parasitic nematodes to prevent them from reaching exceptionally high densities that will be challenging to manage.

Supplementing information generated for plant-parasitic nematodes during this study is the information obtained about the presence of beneficial nematodes in local grain fields. Data emanating from the current study agrees with those from only a few reports for South Africa that beneficial nematode abundance is generally low in local soils where grain crops are produced, e.g., maize (Bekker *et al.*, 2022) and soybean (Mbatyoti *et al.*, 2021; Jansen, 2014). The PV ranges of 47 to 797/200 g soil for bacterivores, 1 to 120/200 g soil for fungivores, 1 to 39/200 g soil for omnivores and zero to 20/200 g soil for predators for survey 2 are in line with those reported by Bekker *et al.* (2022) for maize and by Mbatyoti *et al.* (2021) for soybean. The latter data are in contrast with those by Jansen (2014) for soybean fields (where conventional and genetically-

modified cultivars were grown) and where high numbers of beneficial nematodes prevailed (3501 to 3938 individuals/200 g soil).

The general trend reported that nematode communities in grain fields were dominated by bacterivore genera of the families Acrobelidae, Cephalobidae, and Panagrolaimidae and fungivores of the families Aphelenchidae and Aphelenchoididae generally agrees with data from a maize survey by Bekker *et al.* (2022) and soybean surveys by Jansen (2014) and Mbatyoti *et al.* (2018). It is also in agreement with reports by Djigal *et al.* (2004) for maize in Dakar (Senegal), and Xu *et al.* (2015) for maize in Kunming (China) suggesting that bacterial feeding nematodes are the most abundant metazoans in soil substrates. The lower abundance and occurrence of predators and omnivores in this survey conducted in commercial fields of South African grain producers was not surprising since these two groups are regarded as being very sensitive to soil disturbances (Ferris *et al.*, 2001). Such results also agree with the low abundance and occurrence of these two nematode trophic groups in recent maize (Bekker *et al.*, 2022) and soybean (Mbatyoti *et al.*, 2018; Jansen, 2014) surveys. Since no data on beneficial nematodes have been generated for sunflower and wheat in South Africa, the limited data obtained during this current study for the few fields add value. Research on beneficial nematodes in local grain cropping systems should be expanded and focused on since it will contribute to our understanding of the complex soil-health phenomenon which producers should focus on to enable them to produce crops sustainably.

Although NBI and faunal data were generated only for the second survey of this study, it adds valuable information about the nematode ecosystem status for grain fields. Significant differences in MI 2-5 among the 18 fields sampled are portrayed by a range of values; the lowest MI 2-5 of 2.05 represented by field 3 (4CS) indicates reduced soil ecosystem health and impaired soil processes resulting from soil ecosystem degradation over time (Salamun *et al.*, 2017). This might likely be ascribed to a certain degree to the application of a pesticide; in this case the new-generation nematicide VELUM® 1GR (a.s. Fluopyram). The use of pesticides is typically reported to cause an imbalanced MI 2-5 (Maina *et al.*, 2020). Ploughing most probably also contributed towards the lower MI 2-5 for field 3 (4CS) since it is known to also influence the abundance of soil nematode communities (Lazcano *et al.*, 2021). Conversely, high MI 2-5 values (e.g., field 17JB_3; the highest for this study) are characteristic of a less disturbed soil ecosystem (Salamun *et al.*, 2017) and such environments show gradual increases in maturity and stability of the soil ecosystem (Salamun *et al.*, 2014).

Significant differences were also evident for SI and EI among the 18 fields with those having low to intermediate SI (values of zero to 50) portraying reduced soil food web complexity and shorter food chains (Georgieva *et al.*, 2002; Mohamedova and Lecheva, 2016). Opposed to low SI, the highest SI of 61.69 for field 15 (17JB_3) represented a matured and fertile ecosystem. The lowest EI of 11.65 for field 4 (4CS_2) implies low responsiveness of the opportunistic non-herbivorous

nematode guilds (Ba1 and Fu2) to food resource enrichment, while the opposite is true for field 5 (5JM) with the highest EI of 59.82.

Apart from the MI 2-5, EI and SI that are used as accurate indicators of ecosystem restoration, the primary decomposition channels in the soil food web are regulated by the CI (Ferris *et al.*, 2001; Sánchez-Moreno *et al.*, 2018). Increased CI values indicate a more fungal-dominated pathway with fields 4 (4CS_2) and 11 (14DV) being superior with values of 100. This is an indication of changes in primary decomposition in the soil food webs of these fields over time. Reduced CI values indicate a mainly bacterial-dominated decomposition pathway (Thakur *et al.*, 2019) with field 1 (1SH) representing this pathway the best with the lowest value of 11.83. Increased CI values for most of the fields (10 out of the 18) investigated during this survey might be attributed to changes in the decomposition matter that are supplied to the soil ecosystem (Ferris *et al.*, 2001).

The soils of fields 5 (5JM) and 16 (18JF) represented stressed and enriched conditions due to diminishing nematode community structures as a result of stress conditions, such as limited resources, adverse environmental conditions, and/or recent contamination. Nematode guilds present are those adapted to stress conditions and represent the cp-2 class (Ferris *et al.*, 2001). Opposed to stress conditions, enriched conditions may be ascribed to crop rotation patterns influencing the availability of utilizable resources of nematodes (Zhang *et al.*, 2015b) as well as disturbance of soils and resources becoming available due to mortality of organisms and favourable turnovers/shifts in environmental conditions (Odum, 1985). A flush of microbial activity and enhancement of bacterial-feeding opportunists are associated with such soils (Ferris *et al.*, 2001); the dominating bacterial community is represented by cp-1 class bacterivores, while cp-2 class fungivores constitute a small part of the community. The low SI values in particular in such soils, and those defined as stressed and depleted (discussed below for 15 fields investigated during this study), refer to reduced soil food web complexity and shorter food chains (Mohamedova and Lecheva, 2016; Georgieva *et al.*, 2002).

Only field 15 (17JB_3) represented a matured and fertile faunal profile; indicative of a stable environment containing sufficient enrichment levels to sustain soil organisms. Such soils are highly structured due to higher cp-4 and-5 nematodes, e.g., omnivores, predators, bacterivores and fungivores being present (Zhang *et al.*, 2015a; Ferris *et al.*, 2001). The higher SI value of this field represents a complex nematode community structure, indicative of the existence of multiple trophic links in the soil food web (Maina *et al.*, 2020; Ferris, 2010;). Its low EI, however, implies low responsiveness of the opportunistic non-herbivorous guilds (Ba1 and Fu2) to food resource enrichment; recovery from stress typically occurs in such an ecosystem (Ferris *et al.*, 2001).

Most of the fields sampled (83%), were plotted in the stressed/degraded and depleted quadrat indicating that the quality of these soils is not optimal in terms of the presence of beneficial nematodes as indicated also by low EI and SI values (between zero and 50) (Ferris *et al.*, 2001). Such fields are often associated with management practices such as repeated tillage (Berkelmans

et al., 2003), crop rotation and pesticide and fertilizer application (Carrascosa *et al.*, 2023) which are typical in local grain production areas where the investigated fields are located (Yang *et al.*, 2020; Yan *et al.*, 2018; Mc Donald *et al.*, 2017; Van der Laan *et al.*, 2017; Rusinamhodzi, 2015; Liebenberg, 2012; Derpsch *et al.*, 2010; Hobbs, 2007). These practices rapidly change the trophic distribution in such food webs and adversely impact such low ecosystem structures (Du Preez *et al.*, 2018; Cardoso *et al.*, 2013; Ferris *et al.*, 2001).

None of the fields plotted in Quadrat B (representing a stable and enriched soil ecosystem) represent a stable environment with sufficient enrichment levels to sustain soil organisms and which is highly structured in terms of the occurrence of sensitive nematodes (Zhang *et al.*, 2015a; Ferris *et al.*, 2001). This is an indication of the poor soil health status of grain field producers whose fields were sampled in this study.

No meaningful associations were evident among nematodes (plant-parasitic and beneficial groups) and other parameters such as rainfall, temperature, and the soil-clay% (abiotic) as well as NBIs, crop rotation sequences, dry land and irrigated conditions, and nematicide use. Such associations represent complex interactions for which optimal and specific data points must be collected preferably over longer study periods. Hence this aspect warrants no further discussion since during this study it was only done during one of the two survey studies.

Conclusively, this study accentuated the importance of regular nematode surveys being done. Although this study demonstrated that the dominating plant-parasitic nematodes in the rhizospheres of grain crops are still *Meloidogyne* and *Pratylenchus* as is reported by recent reports, predominant nematode genera in soil showed a shift from those published in literature. The unexpected listing of ectoparasites belonging to the Criconematidae and Dolichodoridae families is hence important and should be taken cognisance of. It is further suggested that *Rotylenchulus* should be investigated further to assess its pathogenicity to grain crops. The addition of beneficial nematode data added value to the scientific domain, but also of our understanding of this component of the nematode community which is becoming more important in terms of using them as bioindicators of soil health.

CHAPTER 5: CONCLUSION

5.1 GENERAL CONCLUSION

The nematode surveys, initiated in response to requests from producers and GrainSA, of 18-grain fields in the Gauteng, North West and Free State provinces, aimed to determine the plant-parasitic nematode and beneficial nematode assemblages in rhizospheres of grain crops grown by local, commercial producers. The first of two objectives was to determine the abundance and diversity of plant-parasitic nematodes (genus and/or species level) and beneficial nematodes (at family level) in each field sampled, and their associations with selected abiotic parameters (rainfall, temperature, soil chemical and physical parameters). The second objective was to identify root-knot nematode species from the 18 fields sampled utilizing classical (morphology and morphometrics) and molecular approaches.

5.2 TESTING OF THE HYPOTHESES

The following hypotheses were tested and are discussed:

i) Plant-parasitic nematode genera that are foreseen to dominate in grain crop rhizospheres are root-knot (*Meloidogyne* Goeldi, 1892) and lesion (*Pratylenchus* Filipjev, 1936) nematodes, while it is expected that beneficial nematode abundance and diversity will be low in such intensified crop ecosystems.

Results from this survey, done during 2020 (survey 1) and 2021 (survey 2) summer growing seasons, showed that root-knot nematodes were the predominant genus present in the roots of fields sampled for survey 1, followed by *Rotylenchulus* Linford and Oliveira, 1940. This agrees with findings by Qwabe (2023), Bekker *et al.* (2022), Pretorius (2018) and Mc Donald *et al.* (2017) that root-knot nematodes dominate in maize fields in South Africa. It also conforms with records that the latter genus is predominant in local soybean production areas as reported by Engelbrecht *et al.* (2021) and Mbatyoti *et al.* (2021). Similarly, it agrees with the limited data generated recently for sunflower by Qwabe (2023). The finding that lesion nematodes followed root-knot nematodes and *Rotylenchulus* in predominance in grain crop roots agrees with findings for maize and soybean in South Africa as reported by Bekker *et al.* (2022), Mbatyoti *et al.* (2021) and Pretorius (2018). Hence the first hypothesis can be partially accepted.

Supplementing information generated for plant-parasitic nematodes during this study is the information obtained about the presence of beneficial nematodes in local grain fields. Data emanating from the current study agrees with those from only a few reports for South Africa that beneficial nematode abundance is generally low in local soils where grain crops are produced, e.g., maize (Bekker *et al.*, 2022) and soybean (Jansen, 2014; Mbatyoti *et al.*, 2021). Since the majority of the fields were plotted in the stressed/degraded and depleted quadrat of the faunal food web, the second part of the hypothesis can be accepted.

ii) The most commonly occurring and predominant root-knot nematode species expected to be identified from the 18 fields of farmers will be *Meloidogyne incognita* (Kofoed and White, 1919) Chitwood, 1949, *Meloidogyne javanica* (Treub, 1885) Chitwood, 1949. The predominant species found infecting maize, soybean, sunflower and wheat crops in the fields sampled during this study, namely, *M. incognita* and *M. javanica* agree with the stated hypothesis and therefore it is accepted. Interestingly, *Meloidogyne arenaria* (Neal, 1889) Cobb, 1890 was also found as the least dominant species agreeing with reports for grain crops by Visagie *et al.* (2018) and Pretorius (2018). The dominance of communities that contained more than one root-knot nematode species opposed to single species populations, from this survey is also in agreement with previous reports for maize and other grain crops grown in South Africa (Qwabe, 2023; Collett, 2020; Rashidifard *et al.*, 2019a, b; Pretorius, 2018; Visagie *et al.*, 2018; Mc Donald *et al.*, 2017). The presence of more than one species in a maize-based production field is, however, an indication that producers might find it a challenge to effectively manage root-knot nematodes in their fields.

5.3 FUTURE RECOMMENDATIONS

i) The present study revealing new information about the ectoparasitic families Dolichodoridae and Criconematidae in the second and third places, respectively, is novel and interesting since it was not reported before. Hence, there is a need for wider-ranging studies to investigate the impact of Dolichodoridae and Criconematidae on grain crops, e.g., its impact on the growth and development, and yield of maize, soybean, sunflower, wheat, and other grain crops grown in rotation in South Africa.

ii) Nematode surveys in grain fields should be done regularly to ensure that accurate information is generated and disseminated regarding the predominant plant-parasitic nematode specie/s as well as the listing of new species since this will impact substantially on the sustainable production of such crops and food security.

iii) The highly efficient molecular technique, SCAR-PCR, could not detect *M. enterolobii* from the 18 fields in this study. Therefore, it could be helpful to use various classical and molecular identification methods when identifying *Meloidogyne* spp., such as those used by Rashidifard *et al.* (2018); namely the NADH5 (nicotinamide adenine dinucleotide+ hydrogen) and genotyping-by-sequencing (GBS), to ensure precise and correct species identification.

iv) More emphasis should be put on obtaining more detailed data on abiotic parameters, and over a longer period, to enable determining associations existing between nematodes and such factors. Although such research is expensive, it should be included in survey studies to enable optimal data generation that will enhance our understanding of the effects of especially the environment and other factors (dry land and irrigation practices; pesticide application, nutrient status, soil type, and others).

REFERENCES

- Abad, P., Castagnone-Sereno, P., Rosso, M., Engler, J. & Favery, B. 2009. Invasion, Feeding and Development. In: Perry, R.N., Moens, M. and Starr, J.L., (Eds). *Root-knot Nematodes*. Wallingford, UK: CAB International Publishing. pp. 163-176.
- Abd-Elgawad, M.M.M. & Askary, T.H. 2015. Impact of phytonematodes on agriculture economy. In: Askary, T.H. and Martinelli, P.R.P., (Eds). *Biocontrol Agents of Phytonematodes*. Giza, Egypt. National Research Centre. pp. 3-49.
- Abebe, E., Mekete, T. & Thomas, W.K. 2011. A critique of current methods in nematode taxonomy. *African Journal of Biotechnology*, 10(3):312-323.
- Adam, M.A.M., Phillips, M.S. & Blok, V.C. 2007. Molecular diagnostic key for identification of single juveniles of seven common and economically important species of root-knot nematode (*Meloidogyne* spp.). *Plant Pathology*, 56(1):190-197.
- Adegbite, A.A. 2011. Reaction of some maize (*Zea mays* L.) varieties to infestation with root-knot nematode, *Meloidogyne incognita* under field conditions. *African Journal of Plant Science*, 5(3):162-167.
- Adisa, O.M., Botai, C.M., Botai, J.O., Hassen, A., Darkey, D., Tesfamariam, E., Adisa, A.F., Adeola, A.M. & Ncongwane, K.P. 2018. Analysis of agro-climatic parameters and their influence on maize production in South Africa. *Theoretical and Applied Climatology*, 134:991-1004.
- Agbenin, N.O. 2011. Biological control of plant parasitic nematodes: prospects and challenges for the poor Africa farmer. A review. *Plant Protection Science*, 47(2):62-67.
- Agenbag, M. 2016. Identification and reproduction potential of South African *Meloidogyne* species. Potchefstroom: North-West University (MSc Dissertation).
- Agricultural Research Council-Small Grain (ARC-SG). 2020. Guideline production of small grains in the summer rainfall region. Available online: <https://www.arc.agric.za/arc-sgi/Documents/2020%20Production%20Guidelines/2020%20Guideline%20for%20the%20production%20of%20small%20grains%20in%20the%20summer%20rainfall%20area.pdf>. Date of Access: 28 Jun. 2021.
- Ahmed, M., Sapp, M., Prior, T., Karssen, G. & Back, M.A. 2016. Technological advancements and their importance for nematode identification. *Soil*, 2(2):257-270.
- Al-Banna, L., Williamson, V. & Gardner, S.L. 1997. Phylogenetic Analysis of Nematodes of the Genus *Pratylenchus* Using Nuclear 26S rDNA. *Molecular Phylogenetics and Evolution*, 7(1):94-102.

- Allen, M.W. & Jensen, H.J. 1951. *Pratylenchus vulnus*, new species (Nematoda Pratylenchinae), a parasite of trees and vines in California. *Proceedings of the Helminthological Society of Washington*, 18(1):47.
- Andrássy, I. 2005. Free-living nematodes of Hungary (Nematoda Errantia). Vol. 1, Hungarian Natural History Museum and Systematic Zoology Research Group of the Hungarian Academy of Sciences, Budapest. pp. 518.
- Andrássy, I. 2009. Free-living nematodes from Albania, including the description of three new species. *Nematologia Mediterranea*, 37(1): 73-88.
- Andrássy, I. & Gibson, J.A. 2007. Nematodes from saline and freshwater lakes of the Vestfold Hills, East Antarctica, including the description of *Hypodontolaimus antarcticus* sp. n. *Polar Biology*, 30:669-678.
- Ashoub, A.H. & Amara, M.T. 2010. Biocontrol activity of some bacterial genera against root-knot nematode, *Meloidogyne incognita*. *Journal of American Science*, 6(10):321-328.
- Asmus, G.L.; Ferraz, L.C.B. & Appezzato de-Gloria, B. 2000. Anatomical changes in corn (*Zea mays* L.) roots caused by *Meloidogyne javanica*. *Nematropica*, 30(1):33-39.
- Atandi, J.G., Haukeland, S., Kariuki, G.M., Coyne, D.L., Karanja, E.N., Musyoka, M.W. & Adamtey, N. 2017. Organic farming provides improved management of plant parasitic nematodes in maize and bean cropping systems. *Agriculture, Ecosystems and Environment*, 247:265-272.
- Aung, T., Windham, G.L. & Williams, W.P. 1990. Reproduction of *Meloidogyne incognita* on open-pollinated maize varieties. *Journal of Nematology*, 22(4):651.
- Back, M.A., Haydock, P.P.J. & Jenkinson, P. 2002. Disease complexes involving plant parasitic nematodes and soilborne pathogens. *Plant Pathology*, 51(6):683-697.
- Bate, R. & Van Rensburg, J.B.J. 1992. Predictive estimation of maize yield loss caused by *Chilo partellus* (Swinhoe) (Lepidoptera: Pyralidae) in maize. *South African Journal of Plant and Soil*, 9(3):150-154.
- Bates, J. A., Taylor, E. J. A., Gans, P. T., & Thomas, J. E. 2002. Determination of relative proportions of *Globodera* species in mixed populations of potato cyst nematodes using PCR product melting peak analysis. *Molecular Plant Pathology*, 3:153-161.
- Bekker, S. 2008. Assessment of the identity, distribution and control options for seed-and leaf-gall nematodes in grass in South Africa. Potchefstroom: North-West University (MSc Dissertation).
- Bekker, S., Fourie, H., Rashidi, M., Daneel, M., Shokoohi, E. & Nel, A. 2016. Discriminating between the eggs of two egg-mass-producing nematode genera using morphometric and molecular techniques. *Nematology*, 18(9):1119-1123.

- Bekker, S., Preez, G.D., Daneel, M. & Fourie, H. 2022. Nematode assemblages in conservation and conventional maize fields: a South African baseline study. *Journal of Plant Diseases and Protection*, 129(5):1235-1248.
- Berkelmans, R., Ferris, H., Tenuta, M. & Van Bruggen, A.H.C. 2003. Effects of long-term crop management on nematode trophic levels other than plant feeders disappear after 1 year of disruptive soil management. *Applied Soil Ecology*, 23(3):223-235.
- Berry, S. D., Fargette, M., Spaull, V. M., Morand, S. & Cadet, P. 2008. Detection and quantification of root-knot nematode (*Meloidogyne javanica*), lesion nematode (*Pratylenchus zeae*) and dagger nematode (*Xiphinema elongatum*) parasites of sugarcane using real-time PCR. *Molecular and Cellular Probes*, 22:168-76.
- Beukes, I., Rose, L.J., Shephard, G.S., Flett, B.C. & Viljoen, A. 2017. Mycotoxigenic *Fusarium* species associated with grain crops in South Africa-A review. *South African Journal of Science*, 113(3-4):1-12.
- Bhardwaj, P. 2019. Types of sampling in research. *Journal of the Practice of Cardiovascular Sciences*, 5(3):157.
- Blok, V. C., Phillips, M. S. & Fargette, M. 1997. Comparison of sequences from the ribosomal DNA intergenic region of *Meloidogyne mayaguensis* and other major tropical root-knot nematodes. *Journal of Nematology*, 29:16-22.
- Blok, V. C., Wishart, J. W., Fargette, M. F., Berthier, K. B. & Phillips, M. S. P. 2002. Mitochondrial DNA differences distinguishing *Meloidogyne mayaguensis* from the major species of tropical root-knot nematodes. *Nematology*, 4:773-781.
- Blok, V.C. 2005. Achievements in and future prospects for molecular diagnostics of plant-parasitic nematodes. *Canadian Journal of Plant Pathology*, 27(2):176-185.
- Blok, V.C. & Powers, T.O. 2009. Biochemical and molecular Identification. In: Perry, R.N., Moens, M. and Starr, J.L., (Eds). *Root-knot nematodes*. Wallingford, UK: CAB International Publishing. pp. 98-118.
- Bolton, C, De Waele, D. & Loots, G.C. 1989. Plant-parasitic nematodes of field crops. 3. Sunflower. *Reviews Nematology*, 12:69-76.
- Bolton, C. & De Waele, D. 1989. Host suitability of commercial sunflower hybrids to *Pratylenchus zeae*. *Journal of Nematology*, 4:682-685.
- Bongers, T. & Bongers, M. 1998. Functional diversity of nematodes. *Applied Soil Ecology*, 10(3):239-251.
- Bongers, T. & Ferris, H. 1999. Nematode community structure as a bioindicator in environmental monitoring. *Trends in Ecology and Evolution*, 14(6):224-228.

- Borlaug, N.E., Narvaez, I., Aresvik, O. & Anderson, R.G. 1969. A green revolution yields a golden harvest. *Columbia Journal World Business*, 4:9-19.
- Bridge, J. & Starr, J.L. 2007. *Plant Nematodes of Agricultural Importance*. Academic Press, Burlington, Massachusetts.
- Brito, J. A., Powers, T. W., Mullin, P. G., Inserra, R. N. & Dickson, D. W. 2004. Morphological and molecular characterization of the *Meloidogyne mayaguensis* isolates from Florida. *Journal of Nematology*, 36:232–240.
- Buys, M. 2022. Evaluation of integrated pest management practices on nematode assemblages in a potato field in South Africa. Potchefstroom: North-West University (PhD Dissertation).
- Byrd, D.W., Kirkpatrick, T. Jr. & Barker, K.R. 1983. An improved technique for cleaning and staining plant tissues for detection of nematodes. *Journal of Nematology*, 15:142-143.
- Cardoso, E.J.B.N., Vasconcellos, R.L.F., Bini, D., Miyauchi, M.Y.H., dos Santos, C.A., Alves, P.R.L., de Paula, A.M., Nakatani, A.S., de Moraes Pereira, J. & Nogueira, M.A. 2013. Soil health: looking for suitable indicators. What should be considered to assess the effects of use and management on soil health? *Agricultural sciences*, 70(4):274-289.
- Carneiro, R.M.D.G. & Almeida, M.R.A. 2001. Electrophoresis technique used in the study of enzymes of gall nematodes for species identification. *Brazilian Nematology*, 25:555–560.
- Carneiro, R.M.D.G., Lima, F.S.D.O. & Correia, V.R. 2017. Methods and tools currently used for the identification of plant parasitic nematodes. *Nematology-Concepts, Diagnosis and Control*. pp. 19. <http://dx.doi.org/10.5772/intechopen.69403>
- Carpenter, A.S., Hiatt, E.E., Lewis, S.A. & Abbott, A.G. 1992. Genomic RFLP analysis of *Meloidogyne arenaria* race 2 populations. *Journal of Nematology*, 24(1):23.
- Carrascosa, A., Pascual, J.A., López-García, Á., Romo-Vaquero, M., De Santiago, A., Ros, M., Petropoulos, S.A. & Alguacil, M.D.M. 2023. Effects of inorganic and compost tea fertilizers application on the taxonomic and functional microbial diversity of the purslane rhizosphere. *Frontiers in Plant Science*, 14.
- Castagnone-Sereno, P., Mulet, K., Danchin, E.G., Koutsovoulos, G.D., Karaulic, M., Da Rocha, M., Bailly-Bechet, M., Pratz, L., Perfus-Barbeoch, L. & Abad, P. 2019. Gene copy number variations as signatures of adaptive evolution in the parthenogenetic, plant-parasitic nematode *Meloidogyne incognita*. *Molecular Ecology*, 28(10):2559-2572.
- Castillo, P. & Vovlas, N. 2007. *Pratylenchus* (Nematoda: Pratylenchidae): diagnosis, biology, pathogenicity and management. In Hunt D. J. and Perry R. N., (Eds). *Nematology monographs and perspectives*, 6. Leiden, The Netherlands: Brill. pp. 1-5.

- Cenis, J.L. 1993. Identification of four major *Meloidogyne* spp. by random amplified polymorphic DNA (RAPD-PCR). *Phytopathology*, 83:76-78.
- Chang, K.F., Hwang, S.F., Conner, R.L., Ahmed, H.U., Zhou, Q., Fu, H., Turnbull, G.D., Nyandoro, R., Strelkov, S.E., McLaren, D.L. & Gossen, B.D. 2018. Effects of *Fusarium avenaceum* and *Rhizoctonia solani* on the growth of soybean in saline soils. *Canadian Journal of Plant Science*, 99(2):128-137.
- Chitwood, D.J. & Perry, R.N. 2009. Reproduction, physiology and biochemistry. In: Perry, R. N., Moens, M. and Starr, J. L., (Eds). *Root-knot nematodes*. Wallingford, UK: CABI International. pp. 182-200.
- Collett, R.L. 2020. A comparative study of the development and reproduction of *Meloidogyne enterolobii* and other thermophilic South African *Meloidogyne* species. Potchefstroom: North-West University (MSc Dissertation).
- Colombo, A., d'Errico, F.P., Giacometti, R, Greco, N. & Roversi, P. 2014. Crop damaging nematodes: From biology to modern control strategies. *Control Methods*. Arena Progetti. Verona, Italy. pp. 93-127.
- Coolen, W.A. & D'herde, C.J. 1972. *A method for the quantitative extraction of nematodes from plant tissue*. State Nematology and Entomology Research Station, Ghent, Belgium. pp 77.
- Coombs, J.T., Michelsen, P.P. & Franco, C.M. 2004. Evaluation of endophytic actinobacteria as antagonists of *Gaeumannomyces graminis* var. *tritici* in wheat. *Biological control*, 29(3):359-366.
- Coyne, D., Nicol, J. & Claudius-Cole, B. 2007. *Practical Plant Nematology: Field and Laboratory Guide*. SP-IPM Secretariat, International Institute of Tropical Agriculture (IITA), Cotonou, Benin.
- Coyne, D.L., Fourie, H. & Moens, M. 2009. Current and future management strategies in resource-poor farming. In: Perry, R.N., Moens, M. and Starr, J.L., (Eds). *Root-knot nematodes*. Wallingford, UK: CAB International Publishing. pp. 444-476.
- Curran, J., Baillie, D., & Webster, J. 1985. Use of genomic DNA restriction fragment length differences to identify nematode species. *Parasitology*, 90(1):137-144.
- Dababat, A.A. & Fourie, H. 2018. Nematode parasites of cereals. In: Sikora R.A, Coyne, D., Hallmann, J. and Timper, P., (Eds). *Plant parasitic nematodes in subtropical and tropical agriculture*. Wallingford, UK: CAB International Publishing. pp.163-221.
- DAFF (Department of Agriculture, Forestry and Fisheries). 2010. Sunflower production guideline. Directorate Agricultural Information Services. Pretoria. Available online: <https://www.nda.agric.za/docs/Brochures/prodGuideSunflower.pdf>. Date of access: 30 Nov. 2021.
- DAFF (Department of Agriculture, Forestry and Fisheries). 2016. Wheat Production Guideline. Directorate Agricultural Information Services. Pretoria. Available online:

<https://www.dalrrd.gov.za/Portals/0/Brochures%20and%20Production%20guidelines/Wheat%20-%20Production%20Guideline.pdf>. Date of access: 30 Nov. 2021.

DAFF (Department of Agriculture, Forestry and Fisheries). 2017. Agricultural remedies that are banned or restricted for use in the Republic of South Africa. Pretoria. Available online: <http://www.thedtic.gov.za/wp-content/uploads/Agric-Remedies.pdf>. Date of access: 30 Nov. 2021.

Davis, E.L. & MacGuidwin, A.E. 2000. *Lesion nematode disease*. Plant Health instructor. <http://doi:10.1094/PHI-I-2000-1030-02>

De Man, J.G. 1880. The natives, nematodes living freely in the pure earth and in the fresh water Preliminary report and descriptive-systematic theil. *Journal of the Dutch Zoological Association* 5:1-104.

De Waele, D. & Elsen, A. 2002. Migratory endoparasites: *Pratylenchus* and *Radopholus* species. In: Starr, J.L., Cook, R. and Bridge, J., (Eds). *Plant resistance to parasitic nematodes*. Wallingford, UK: CABI Publishing. pp.175-206.

De Waele, D. & Jordaan, E.M. 1988. Plant-parasitic nematodes on field crops in South Africa. 1. Maize. *Journal of Nematology*, (1):65-74.

De Waele, D., Mc Donald, A.H. & De Waele, E. 1988. Influence of seaweed concentrate on the reproduction of *Pratylenchus zeae* (Nematoda) on maize. *Nematologica* 34:71–77.

De Grisse, A.T. 1963. A counting dish for nematodes excluding border effect. *Nematologica*, 9:162.

Decraemer, W. & Hunt, D.J. 2013. Structure and classification. In Perry, R.N. and Moens, M., (Eds). *Plant nematology*, 2nd edn. Wallingford: CAB International Publishing. pp. 3-39.

Derpsch, R., Friedrich, T., Kassam, A. & Hong-wen, L. 2010. Current status of adoption of no-till farming in the world and some of its main benefits. *International Journal of Agricultural and Biological Engineering*, 3:1-25.

Derycke, S., Vanaverbeke, J., Rigaux, A., Backeljau, T. & Moens, T. 2010. Exploring the use of cytochrome oxidase c subunit 1 (COI) for DNA barcoding of free-living marine nematodes. *PloS one*, 5:e13716. <https://doi.org/10.1371/journal.pone.0013716>.

Dewar, A.M., Haylock, L.A., May, M.J., Beane, J. & Perry, R.N. 2000. Glyphosate applied to genetically modified herbicide-tolerant sugar beet and 'volunteer'potatoes reduces populations of potato cyst nematodes and the number and size of daughter tubers. *Annals of Applied Biology*, 136(3):179-187.

Dias-Arieira, C.R., de Araújo, F.G., Kaneko, L. & Santiago, D.C. 2018. Biological control of *Pratylenchus brachyurus* in soya bean crops. *Journal of Phytopathology*, 166:722–728.

- Djigal, D., Brauman, A., Diop, T.A., Chotte, J.L. & Villenave, C. 2004. Influence of bacterial-feeding nematodes (Cephalobidae) on soil microbial communities during maize growth. *Soil Biology and Biochemistry*, 36(2):323-331.
- Dlamini, T.S., Magingxa, L. & Liebenberg, F. 2015. Estimating the economic value of the national cultivar trials in South Africa: A case for sorghum, sunflower, soybeans and dry beans. Paper presented at: The Fourth Congress of the Italian Association of Agricultural and Applied Economics (AIEAA); June 11–12; Ancona, Italy.
- Dlamini, T.S., Tshabalala, P. & Mutengwa, T. 2014. Soybeans production in South Africa OCL, 21(2): D207.
- Doran, J.W. & Safley, M. 1997. Defining and assessing soil health and sustainable productivity. In: Pankhurst, C.E., Doube, B.M. and Gupta, V.V.S.R., (Eds). *Biological indicators of soil health*. New York: CAB International Publishing. pp. 1-22.
- Dos Santos, M.F.A., Mattos, W.S., Monteiro, J.M.S., Almeida, M.R.A, Jorge, A.S. Jr., Cares, J.E., Castagnones-Sereno, P., Coyne, D. & Carnero, R.M.D.G. 2019. Diversity of *Meloidogyne* spp. From peri-urban areas of sub-Saharan Africa and their genetic similarity with populations from Latin America. *Physiological and Molecular Plant Pathology*, 105:110-118. <https://doi.org/10.1016/j.pmp.2018.08.004>
- Du Plessis, J. 2003. Maize production. Pretoria: Department of Agriculture. Available online: <http://www.arc.agric.za/arc-gci/Fact%20Sheets%20Library/Maize-infopak.pdf>. Date of access: 13 Jun. 2021.
- Du Preez, C.C. & Van Huyssteen, C.W. 2020. Threats to soil and water resources in South Africa. *Environmental research*, 183:1090
- Du Preez, C.C., Kotzé, E. & Van Huyssteen, C.W. 2018. Southern African soils and their susceptibility to degradation. In: Holmes, P.J. and Boardman, J., (Eds). *Southern African landscapes and environmental change*. London: Routledge. Pp. 29-52.
- Du Preez, G., Daneel, M., De Goede, R., Du Toit, M.J., Ferris, H., Fourie, H., Geisen, S., Kakouli-Duarte, T., Korthals, G., Sánchez-Moreno, S. & Schmidt, J.H. 2022. Nematode-based indices in soil ecology: Application, utility, and future directions. *Soil Biology and Biochemistry*, 169:108640.
- Duru, M., Therond, O., Martin, G., Martin-Clouaire, R., Magne, M.-A., Justes, E. & Bergez, J.-E. 2015. How to implement biodiversity-based agriculture to enhance ecosystem services-a review. *Agronomy for sustainable development*, 35(4):1259-1281.
- Eisenback, J.D. 1985. Diagnostic characters useful in the identification of four most common species of root-knot nematodes (*Meloidogyne* spp.). In: Sasser, J.N. and Carter, C.C., (Eds). *An advanced treatise on Meloidogyne vol. 1. Biology and control*. Raleigh, NC, USA, North Carolina State University Graphics. Pp. 95-112.

- Elhady, A., Heuer, H. & Hallmann, J. 2018. Plant parasitic nematodes on soybean in expanding production areas of temperate regions. *Journal of Plant Diseases and Protection*, 125:567-576.
- Elshire, R.J., Glaubitz, J.C., Sun, Q., Poland, J.A., Kawamoto, K., Buckler, E.S. & Mitchell, S.E. 2011. A robust, simple genotyping-by-sequencing (GBS) approach for high diversity species. *PLoS one*, 6(5):e19379.
- Engelbrecht, G., Claassens, S., Mienie, C.M. & Fourie, H. 2020. South Africa: an important soybean producer in sub-Saharan Africa and the quest for managing nematode pests of the crop. *Agriculture*, 10(6):242.
- Engelbrecht, G., Claassens, S., Mienie, C.M. & Fourie, H. 2021. Screening of rhizosphere bacteria and nematode populations associated with soybean roots in the Mpumalanga Highveld of South Africa. *Microorganisms*, 9(9):1813.
- Engelbrecht, G., Horak, I., Jansen van Rensburg, P.J. & Claassens, S. 2018. Bacillus-based bionematicides: Development, modes of action and commercialisation. *Biocontrol of Science Technology*, 28:629–653.
- Escobar, C., Barcala, M., Cabrera, J. & Fenoll, C. 2015. Overview of root-knot nematodes and giant cells. In: Escobar, C. and Fenoll, C., (Eds). *Plant nematode interactions - a view on compatible interrelationships: vol 73. Advances in botanical research*. Oxford, UK: Elsevier. pp. 1-32.
- Esmeraldo, M.Q. 2017. *Effects of tillage practices on some key soil parameters: A case study in the KwaZulu-Natal Midlands, South Africa*. Stellenbosch: Stellenbosch University (MSc Dissertation).
- Evans, K. & Haydock, P.P.J. 1993. Interactions of nematodes with root-rot fungi. In: Khan, M.W., (Eds). *Nematode interactions*. Dordrecht: Springer. pp.104-133.
- FAO (Food and Agriculture Organization of the United Nations). 2008. Conservation agriculture. Available online: <https://www.fao.org/conservation-agriculture/en/>. Date of access: 28 Aug. 2021.
- FAO. 2014. What is conservation agriculture? Available online: <http://www.fao.org/ag/ca/1a.html> Date of access: 06 Jun. 2023.
- Fargette, M., Lollier, V., Phillips, M., Blok, V. & Frutos, R. 2005. AFLP analysis of the genetic diversity of *Meloidogyne chitwoodi* and *M. fallax*, major agricultural pests. *Biology reports*, 328(5):455-462.
- Ferris, H. & Bongers, T. 2009. Indices developed specifically for analysis of nematode assemblages. In: Wilson, M. and Kakouli-Duarte, T., (Eds). *Nematodes as Environmental Indicators*. Wallingford, UK: CAB International Publishing. pp.124-145.

- Ferris, H., 2010. Form and function: Metabolic footprints of nematodes in the soil food web. *European Journal of Soil Biology*, 46:97-104.
- Ferris, H., Bongers, T. & de Goede, R.G. 2001. A framework for soil food web diagnostics: extension of the nematode faunal analysis concept. *Applied Soil Ecology*, 18(1):13-29.
- Ferris, H., Goodell, P. & McKenry, M. 1981. Sampling for nematodes. *California Agriculture*, 35(5):13-15.
- Ferris, H., Sánchez-Moreno, S. & Brennan, E.B. 2012. Structure, functions and interguild relationships of the soil nematode assemblage in organic vegetable production. *Applied Soil Ecology*, 61:16-25.
- Fischer, R., Bohn, F., de Paula, M.D., Dislich, C., Groeneveld, J., Gutiérrez, A.G., Kazmierczak, M., Knapp, N., Lehmann, S., Paulick, S. & Pütz, S. 2016. Lessons learned from applying a forest gap model to understand ecosystem and carbon dynamics of complex tropical forests. *Ecological Modelling*, 326:124-133.
- Foley, R.C., Kidd, B.N., Hane, J.K., Anderson, J.P. & Singh, K.B. 2016. Reactive Oxygen Species Play a Role in the Infection of the Necrotrophic Fungi, *Rhizoctonia solani* in Wheat. *PloS one*, 11(3): e0152548.
- Fourie, H. & Mc Donald, A.H. 2001. Report for Project M151/60: Chemical Control Options for Plant-Parasitic Nematodes Associated with Soybean in South Africa; Agricultural Research Council—Grain Crops Institute: Potchefstroom, South Africa.
- Fourie, H. & Mc Donald, A.H. 2007. Chemical control options for plant-parasitic nematodes associated with soybean in South Africa. Report on project M151/60. Agricultural Research Council—Grain Crops Institute (ARC-GCI), Potchefstroom.
- Fourie, H., Ahuja, P., Lammers, J. & Daneel, M. 2016. Brassicacea-based management strategies as an alternative to combat nematode pests: A synopsis. *Crop Protection*, 80:21-41.
- Fourie, H., De Waele, D., Mc Donald, A.H., Marais, M. & De Beer, A. 2015. Nematode pests threatening soybean production in South Africa, with reference to *Meloidogyne*. *South African Journal of Science*, 111:1-9.
- Fourie, H., Mc Donald, A.H. & De Waele, D. 2006. Host suitability of South African and foreign soybean cultivars to *Meloidogyne incognita* race 2. *South African Journal of Plant Soil*, 23:132-137.
- Fourie, H., Mc Donald, A.H. & Loots, G.C. 2001a. Plant-parasitic nematodes in field crops in South Africa 6: Soybean. *Nematology*, 3:447-454.
- Fourie, H., Mc Donald, A.H., Steenkamp, S. & De Waele, D. 2017. Nematode Pests of Leguminous and Oilseed Crops. In Fourie, H., Spaull, V.W., Jones, R.K., Daneel, M.S., De Waele, D., (Eds).

- Nematology in South Africa: A View from the 21st Century*. Cham, Switzerland: Springer International Publishing. pp. 201-210. https://doi.org/10.1007/978-3-319-44210-5_9
- Fourie, H., Mothata, T.S., Ntidi, K.N. & De Waele, D. 2012. Indications of variation in host suitability to root-knot nematode populations in commercial tomato varieties. *African Journal of Agricultural Research*, 7: 2344-2352.
- Fourie, H., Van Aardt, W.J., Venter, C. & Tiedt, L.R. 2014. The effects of CropGuard® on the motility, ultrastructure, and respiration of two *Meloidogyne* species. *Nematropica*, 44(1):85-92.
- Fourie, H., Zijlstra, C. & Mc Donald, A.H. 2001b. Identification of root-knot nematode species occurring in South Africa using the SCAR-PCR technique. *Journal of Nematology*, 3:675-680.
- Gao, H., Qi, G., Yin, R., Zhang, H., Li, C. & Zhao, X. 2016. *Bacillus cereus* strain S2 shows high nematocidal activity against *Meloidogyne incognita* by producing sphingosine. *Science Republic*, 6:605.
- Georgieva, S.S., McGrath, S.P., Hooper, D.J. & Chambers, B.S. 2002. Nematode communities under stress: the long-term effects of heavy metals in soil treated with sewage sludge. *Applied soil Ecology*, 20(1):27-42.
- Ghaderi, R. & Karssen, G. 2020. An updated checklist of *Meloidogyne* Göldi, 1887 species, with a diagnostic compendium for second-stage juveniles and males. *Journal of Crop Protection*, 9(2):183-193.
- Gheysen, G. & Mitchum, M.G. 2011. How nematodes manipulate plant development pathways for infection. *Current Opinion in Plant Biology*, 14(4):415-421.
- Giller, K.E., Hijbeek, R., Andersson, J.A. & Sumberg, J. 2021. Regenerative agriculture: an agronomic perspective. *Outlook on agriculture*, 50(1):13-25.
- Girgan, C., Claassens, S. & Fourie, H. 2020a. Nematode assemblages and soil microbial communities in soils associated with glyphosate-resistant soybean. *South African Journal of Plant and Soil*, 37(1):11-22.
- Girgan, C., du Preez, G., Marais, M., Swart, A. & Fourie, H. 2020b. Nematodes and the effect of seasonality in grassland habitats of South Africa. *Journal of Nematology*, 52. <https://doi.org/10.21307/jofnem-2020-116>.
- Goergen, G., Kumar, P.L., Sankung, S.B., Togola, A. & Tamò, M. 2016. First report of outbreaks of the fall armyworm *Spodoptera frugiperda* (JE Smith) (Lepidoptera, Noctuidae), a new alien invasive pest in West and Central Africa. *PloS one* 11(10):e0165632.
- Goswami, B.K. & Agarwal, D.K. 1978. Interrelationships between species of *Fusarium* and root-knot nematode, *Meloidogyne incognita* in soybean. *Nematologia Mediterranea*, 6:125-128.

- Gotosa, J., Kodzwa, J., Gwenzi, W. & Nyamangara, J. 2021. Maize nitrogen uptake and productivity under reduced and conventional tillage. *Nutrient Cycling in Agroecosystems*, 119(1):23-36.
- Gouse, M. 2012. GM maize as subsistence crop: The South African smallholder experience. *AgBioForum*, 15(2):163-174.
- Gouse, M., Sengupta, D., Zambrano, P. & Zepeda, J.F. 2016. Genetically modified maize: less drudgery for her, more maize for him? Evidence from smallholder maize farmers in South Africa. *World Development*, 83:27-38.
- Grain South Africa. 2021a. Grain market overview. Available online: <http://www.grainsa.co.za>. Date of access: 08 Jun. 2021.
- Grain South Africa. 2021b. Conservation Agriculture. Available online: <https://www.grainsa.co.za/grainresearch/conservation-agriculture> Date of access: 20 July 2021.
- Greco, N. & Vito, M.D. 2009. Population dynamics and damage levels. In: Perry, R. N., Moens, M. and Starr, J. L., (Eds). *Root-knot nematodes*. Wallingford, UK: CABI International. pp. 246-274. doi: 10.1079/9781845934927.0246
- Gruzdeva, L., Matveeva, E. & Kovalenko, T. 2007. Changes in soil nematode communities under the impact of fertilizers. *Eurasian Soil Science*, 40:681-693.
- Hallmann, J. & Subbotin, S.A. 2018. Methods for extraction, processing and detection of plant and soil nematodes. In: Sikora, R.A., Coyne, D., Hallmann, J. and Timper, P., (Eds). *Plant Nematodes in Subtropical and Tropical Agriculture*. Wallingford, UK: CAB International Publishing. pp. 87-119.
- Hartman, G.L., Haudenschild, J., Smith, K., Tooley, P., Shelton, J., Bulluck, R., Engle, J.S., Magarey, R., Royer, M., Sutker, E. & Cardwell, K. 2009. Recovery plan for red leaf blotch of soybean caused by *Phoma glycinicola*. *Government Publication/Report*. pp. 4-21. Available online: <https://www.ars.usda.gov/research/publications/publication/?seqNo115=242622> Date of access: 27 Jul. 2022.
- Hartman, G.L., McCormick, S.P. & O'Donnell, K. 2019. Trichothecene-Producing *Fusarium* species isolated from soybean roots in Ethiopia and Ghana and their pathogenicity on soybean. *Plant Diseases*, 103:2070-2075.
- Hassan, J., Chishti, M.Z., Rasheed, M., Ahmad, I., Ahmad, F. & Lone, B. 2009. Nematodes associated with *Zea mays* and their control through organic soil amendments. *International Journal of Plant Production*, 3:71-76.
- Haydock, P.P.J., Woods, S.R., Grove, I.G. & Hare, M.C. 2013. Chemical control of nematodes. In: Perry, R.N. and Moens, M., (Eds). *Plant Nematology*, 2nd edn. Wallingford: CAB International Publishing. pp. 459-479.

- Heinrichs, E.A. & Muniappan, R. 2018. Integrated Pest Management for Tropical Crops: Soybeans. *CAB Reviews*, 13(055):1-44.
- Heyns, J. 1971. A guide to the plant and soil nematodes of South Africa. *A guide to the plant and soil nematodes of South Africa*. Cape Town: A.A Balkema. pp. 233.
- Hobbs, P. 2007. Conservation agriculture: what is it and why is it important for future sustainable food production? *The Journal of Agricultural Science*, 145.
- Holterman, M. M., Oggenfuss, M., Frey, J. E. & Kiewnick, S. 2012. Evaluation of high-resolution melting curve analysis as a tool for root-knot nematode diagnostics. *Phytopathology*, 160:59-66.
- Hugo, H.J., Mouton, C. & Malan, A.P. 2014. Accelerated microbial degradation of nematicides in vineyard and orchard soils. *South African Journal of Enology and Viticulture*, 35(1):157-167.
- Hunt, D.J. & Handoo, Z.A. 2009. Taxonomy, identification and principal species. In: Perry, R.N., Moens, M. and Starr, J.L., (Eds). *Root-knot Nematodes*. Wallingford, UK: CAB International Publishing. pp. 55-88.
- Hunt, D.J. & Handoo, Z.A. 2012. Root-knot nematodes. In: Manzanilla-López, R.H. and Marbán-Mendoza, N., (Eds). *Practical Plant Nematology*. Basic Library of Agriculture, Guadalajara, Mexico, pp. 359–410.
- Ibrahim, A., Al-Hazmi, A., Al-Yahya, F. & Alderfasi, A. 1999. Damage potential and reproduction of *Heterodera avenae* on wheat and barley under Saudi field conditions. *Nematology*, 1(6):625-630.
- Ibrahim, S. K., Perry, R. N., Burrows, P. R. & Hooper, D.J. 1994. Differentiation of species and populations of *Aphelenchoides* and of *Ditylenchus angustus* using a fragment of ribosomal DNA. *Journal of Nematology*, 26:412-421.
- Ibrahim, S.K., Perry, R.N. & Webb, R.M. 1995. Use of isoenzyme and protein phenotypes to discriminate between six *Pratylenchus* species from Great Britain. *Annals of Applied Biology*, 126(2):317-327.
- Ingram, J. 2011. A food systems approach to researching food security and its interactions with global environmental change. *Food Security*, 3(4):417-431.
- Jackson-Ziems, T. A. 2016. "Root-Lesion Nematodes". In: Harveson R. M., Markell, S. G., Block, C. C. and Gulya, T. J., (Eds). *Compendium of Sunflower Diseases and Pests*. St. Paul: APS Press. pp. 71-73.
- Jacobsen, C.S. & Hjelmsø, M.H. 2014. Agricultural soils, pesticides and microbial diversity. *Current Opinion in Biotechnology*, 27:15-20.
- Jamal, Q., Cho, J.Y., Moon, J.H., Munir, S., Anees, M. & Kim, K.Y. 2017. Identification for the First Time of Cyclo (d-Pro-I-Leu) Produced by *Bacillus amyloliquefaciens* Y1 as a Nematocide for

Control of *Meloidogyne incognita*. *Molecules: A Journal of Synthetic Chemistry and Natural Product Chemistry*, 22(11).

James, C. 2015. Global status of commercialized biotech/GM crops: 2015. ISAAA brief No. 51. Available online: <http://www.isaaa.org/resources>. Date of Access: 11 Jun. 2022.

Jansen, C. 2014. *Microbial community structure and nematode diversity in soybean-based cropping systems*. Potchefstroom: North-West University (MSc Dissertation).

Jansen-Girgan, C., Claassens, S. & Fourie, H. 2016. *In vitro* evaluations to determine the effect of *Bacillus firmus* strains on the motility of *Meloidogyne incognita* second-stage juveniles. *Tropical Plant Pathology*, 41:320-324.

Janssen, T., Karssen, G., Verhaeven, M., Coyne, D. & Bert, W. 2016. Mitochondrial coding genome analysis of tropical root-knot nematodes (*Meloidogyne*) supports haplotype-based diagnostics and reveals evidence of recent reticulate evolution. *Scientific Reports*, 6(22591):1-13. DOI: 10.1038/srep22591.

Jaraba-Navas, J., Rothrock, C. S. & Kirkpatrick, T. L. 2007. Influence of the soil texture on the interaction between *Meloidogyne incognita* and *Thielaviopsis basicola* on cotton. *Phytopathology*, 97:S51.

Jarvey, J.A. 2009. A review on soybean rust from a South African perspective. *South African Journal of Soil Science*, 105:103-108.

Jayne, T. S., Chamberlin, J. & Benfica, R. 2018. Africa's unfolding economic transformation. *The Journal of Development Studies*, 54(5): 777-787.

Jepson, S.B. 1987. *Identification of root-knot nematodes (Meloidogyne species)*. Commonwealth Agricultural Bureaux, Farnham Royal, UK.

Jones, J.T., Haegeman, A., Danchin, E.G., Gaur, H.S., Helder, J., Jones, M.G., Kikuchi, T., Manzanilla-López, R., Palomares-Rius, J.E., Wesemael, W.M. & Perry, R.N. 2013. Top 10 plant-parasitic nematodes in molecular plant pathology. *Molecular Plant Pathology*, 14(9):946-961.

Jones, R.K. 2017. Nematode control and nematicides: developments since 1982 and future trends. In Fourie, H., Spaull, V.W., Jones, R.K., Daneel, M.S. and De Waele, D., (Eds). *Nematology in South Africa: A view from the 21st century*. Cham, Switzerland: Springer International Publishing. pp.129-150.

Jordaan, E.M. & De Waele, D. 1988. Effects of four herbicides on the infestation of maize by the nematode *Pratylenchus zeae*. *South African Journal of Plant and Soil*, 5(3):165-165.

Jordaan, E.M., Loots, G.C., Jooste, W.J. & De Waele, D. 1987. Effects of Root-Lesion Nematodes (*Pratylenchus brachyurus* Godfrey and *P. Zeae* Graham) and *Fusarium moniliforme* Sheldon Alone or in Combination, On Maize. *Nematologica*, 33(2):213-219.

- Jordaan, E.M., Van den Berg, E. & De Waele, D. 1992. Plant-parasitic nematodes of field crops in South Africa. 5. Wheat. *Fundamental and Applied Nematology*, 15:531-537.
- Ju, Y., Lin, Y., Yang, G., Wu, H. & Pan, Y. 2019. Development of recombinase polymerase amplification assay for rapid detection of *Meloidogyne incognita*, *M. javanica*, *M. arenaria*, and *M. enterolobii*. *European Journal of Plant Pathology*, 155:1155-1163.
- Kagoda, F., Hearne, S., Adewuyi, O. & Coyne, D.L. 2015. Response of drought tolerant maize inbreds to water stress under nematode infested conditions. *Euphytica*, 206(1):77-87.
- Karssen, G. & Moens, M. 2006. Root knot nematodes. In: Perry, R. N. and Moens, M. (Eds). *Plant Nematology*. Wallingford, UK: CABI Publishing. pp. 59-90.
- Karssen, G., Wesemael, W. & Moens, M. 2013. Root-knot nematodes. In: Perry, R.N., and Moens, M., (Eds). *Plant Nematology*, 2nd edn. Wallingford, UK: CAB International Publishing. pp. 73-108.
- Keetch, D.P. & Buckley, N.H. 1984. A checklist of the plant-parasitic nematodes of Southern Africa. Technical communication No 195. South African Department of Agriculture Technical Services, Pretoria. pp. 213.
- Khojely, D.M., Ibrahim, S.E., Sapey, E. & Han, T. 2018. History, current status, and prospects of soybean production and research in sub-Saharan Africa. *The Crop Journal*, 6(3):226-235.
- 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.
- Kleynhans, K.P.N. 1991. The root-knot nematodes of South Africa. Technical Communication No. 231. Department of Agricultural Development, Pretoria.
- Kopittke, P.M., Menzies, N.W., Wang, P., McKenna, B.A. v Lombi, E. 2019. Soil and the intensification of agriculture for global food security. *Environment International* 132:105078.
- Kriel, G. 2019. Learning about regenerative agriculture. Available online: <https://www.farmersweekly.co.za/crops/field-crops/Learning-about-regenerative-agriculture/>. Date of access 16 Sep. 2022.
- Lazcano, C., Deniston-Sheets, H.M., Stubler, C., Hodson, A.K., Watts, K.R., Afriyie, P., Casassa, L.F. & Peterson, J.C.D. 2021. Soil management induced shifts in nematode food webs within a Mediterranean vineyard in the Central Coast of California (USA). *Applied Soil Ecology*, 157:103756.
- Lee, Y.S. & Kim, K.Y. 2016. Antagonistic potential of *Bacillus pumilus* L1 against root-knot nematode, *Meloidogyne arenaria*. *Journal of Phytopathology*, 164:29–39.
- Liebenberg, A. J. 2012. Soybean Production Manual. Agricultural Research Council-Grain Crops Institute. Potchefstroom, South Africa. pp. 159.

- Lima, F.S.D.O., Santos, G.R.D., Nogueria, S.R, Santos, P.R.R.D. & Correa, V.R. 2015. Population dynamics of the root lesion nematode, *Pratylenchus brachyurus*, in soybean fields in Tocantins State and its effect to soybean yield. *Nematropica*, 45:170-177.
- Liu, A. & Burke, J.M. 2006. Patterns of nucleotide diversity in wild and cultivated sunflower. *Genetics*, 173(1):321-330.
- Lizaso, J.I., Ruiz-Ramos, M., Rodríguez, L., Gabaldon-Leal, C., Oliveira, J.A., Lorite, I.J., Sánchez, D., García, E. & Rodríguez, A. 2018. Impact of high temperatures in maize: Phenology and yield components. *Field Crops Research*, 216:29-140.
- Loggenberg, A. 2021. Measuring ecological restoration in conservation agricultural fields in the eastern Free State (South Africa) using nematodes and other soil bioindicators. Potchefstroom: North-West University (MSc Dissertation).
- Long, H., Lui, H., Yan, X.N. & Xu, J.H. 2006. Development of a PCR diagnostic for the root-knot nematode *Meloidogyne enterolobii*. *Acta Phytopathology Sinica*, 36(2):109-115.
- Loof, P.A. 1960. Taxonomic studies on the genus *Pratylenchus* (Nematoda) With a summary: Taxonomic studies on the nematode genus *Pratylenchus*. *Journal of Plant Diseases*, 66(2):29-90.
- Lopes, E.A., Dallemole-Giaretta, R., dos Santos Neves, W., Parreira, D.F. & Ferreira, P.A. 2019. Eco-friendly approaches to the management of plant-parasitic nematodes. *Plant Health Under Biotic Stress: Volume 1: Organic Strategies*. pp.167-186.
- Lopez-Nicora, H.D., Craig, J.P., Gao, X., Lambert, K.N. & Niblack, T.L. 2012. Evaluation of cultivar resistance to soybean cyst nematode with a quantitative polymerase chain reaction assay. *Plant Disease*, 96(10):1556-1563.
- Maafi, Z.T., Subbotin, S. & Moens, M. 2003. Molecular identification of cyst-forming nematodes (Heteroderidae) from Iran and a phylogeny based on ITS-rDNA sequences. *Nematology*, 5(1):99-111.
- Máali, S. 2017. Delayed planting impacts sunflower yield. Available online: <https://www.grainsa.co.za/delayed-planting-impacts-sunflower-yield>. Date of access: 23 Jun. 2021.
- Machado, A.C.Z. & Araújo Filho, J.V. 2016. Broad-sense heritability and variance component estimates for *Pratylenchus brachyurus* resistance in Brazilian soybean genotypes. *Tropical Plant Pathology*, 41:390-396.
- Maina, S., Karuri, H. & Ng'endo, R.N. 2019. Nematode soil food webs in maize agro-ecosystems and their implication on plant-parasitic nematodes. *Phytoparasitica*, 47:743-758.
- Maina, S., Karuri, H. & Ng'endo, R.N. 2020. Nematode metabolic footprints, ecological and functional indices in tropical maize-beans agro-ecosystems under different farming practices. *Acta Oecologica*, 108.

- Mamabolo, E., Makwela, M.M. & Tsilo, T.J. 2020. Achieving sustainability and biodiversity conservation in agriculture: importance, challenges and prospects. *European Journal of Sustainable Development*, 9(3):616-616.
- Mao, X., Hu, F., Griffiths, B. & Li, H. 2006. Bacterial-feeding nematodes enhance root growth of tomato seedlings. *Soil Biology and Biochemistry*, 38(7):1615-1622.
- Marais, M. 2002. Identification Job Sheet N3092: Dataset from South African Plant-Parasitic Nematode Survey Database; Nematology Unit, Biosystematics Division, Plant Protection Research Institute, Agricultural Research Council: Pretoria, South Africa, 2012. *Eastern Nigeria Journal Sustainable Agricultural*, 20:101-110.
- Marais, M. 2006. South African plant-parasitic nematode survey (SAPPNS), *Plant Protection News*, 67:6.
- Marais, M., Swart, A., Fourie, H., Berry, S.D., Knoetze, R. & Malan, A. 2017. Techniques and Procedures. In: Fourie, H., Spaull, V.W., Jones, R.K., Daneel, M.S. and De Waele, D., (Eds). *Nematology in South Africa: A view from the 21st Century*. Cham, Switzerland: Springer International Publishing. pp. 73-118.
- Matlala, F.L. 2020. Population dynamics of *Meloidogyne* spp. and the effect of pre-plant practices on the pest in tomato net houses in South Africa. Potchefstroom: North-West University (MSc Dissertation)
- Mbatyoti, A., Daneel, M.S., Swart, A., De Waele, D. & Fourie, H. 2018. Terrestrial non-parasitic nematode assemblages associated with glyphosate-tolerant and conventional soybean-based cropping systems. *Journal of Nematology*, 50(3):243-260.
- Mbatyoti, A., Daneel, M.S., Swart, A., Marais, M., De Waele, D. & Fourie, H. 2020. Plant-parasitic nematode assemblages associated with glyphosate tolerant and conventional soybean cultivars in South Africa. *African Zoology*, 55(1):93-107.
- Mbatyoti, A., De Beer, A., Daneel, M.S., Swart, A., Marais, M., De Waele, D. & Fourie, H. 2021. The host status of glyphosate-tolerant soybean genotypes to *Meloidogyne incognita* and *Pratylenchus infection*. *Tropical Plant Pathology*, 46:336-349.
- Mc Donald, A.H. & De Waele, D. 1987a. Control strategies against nematode pests on maize: a literature review. Technical Communication No. 212. Department of Agriculture, Pretoria. pp. 55-60.
- Mc Donald, A.H. & De Waele, D. 1987b. Effect of two nematicides on nematode populations associated with maize. *Phytophylactica*, 19:475-478.
- Mc Donald, A.H. & Nicol, J. 2005. Nematode parasites of cereals. In: Luc, M., Sikora, R.A. and Bridge, J., (Eds). *Plant parasitic nematodes in subtropical and tropical agriculture*, 2nd edn. Wallingford, UK: CAB International Publishing. pp. 131-191.

- Mc Donald, A.H., De Waele, D. & Fourie, H. 2017. Nematode Pest of Maize and Other Cereal Crops. In: Fourie, H., Spaull, V.W., Jones, R.K., Daneel, M.S. and De Waele, D., (Eds). *Nematology in South Africa: A view from the 21st Century*. Cham, Switzerland: Springer International Publishing. pp. 183-199.
- Mcsorley, R. 2011. Overview of organic amendments for management of plant-parasitic nematodes, with case studies from Florida. *Journal of Nematology*, 43(2):69.
- Miamoto, A., Silva, M.T.R.E., Dias-Arieira, C.R. & Puerari, H.H. 2017. Alternative products for *Pratylenchus brachyurus* and *Meloidogyne javanica* management in soya bean plants. *Journal of Phytopathology*, 165(10):635-640.
- Mienie, C.M.S., Fourie, H., Smit, M.A., Van Staden, J. & Botha, F.C. 2002. Identification of AFLP markers in soybean linked to resistance to *Meloidogyne javanica* and conversion to sequence characterized amplified regions (SCARS). *Plant Growth Regulation* 37:157–166. <http://dx.doi.org/10.1023/A:1020585023976>.
- Moens, M. & Perry, R.N. 2009. Migratory plant endoparasitic nematodes: a group rich in contrasts and divergence. *Annual Review of Phytopathology*, 47:313-332.
- 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., and Starr, J.L., (Eds). *Root-knot nematodes*. Wallingford: CAB International Publishing. pp. 1-17.
- Mohamedova, M.S. & Lecheva, I. 2016. Soil nematode response to heavy metal pollution of industrial origin in Bulgaria. *Turkish Journal of Agriculture and Forestry*, 3(2):143-150.
- Mokrini, F., Moens, M. & Viaene, N. 2016. Root-lesion and cyst nematodes in cereal fields in Morocco: Species identification, population diversity and crop resistance. Ghent University, Faculty of Bioscience Engineering, Ghent, Belgium. (PhD Thesis).
- Mokrini, F., Waeyenberge, L., Viaene, N. & Moens, M. 2012. First report of the cereal cyst nematode *Heterodera latipons* on wheat in Morocco. *Plant Disease*, 96(5):774-774.
- Mokrini, F., Waeyenberge, L., Viaene, N., Abbad Andaloussi, F. & Moens, M. 2013. Quantitative detection of the root-lesion nematode, *Pratylenchus penetrans*, using qPCR. *European Journal of Plant Pathology*, 137:403-413.
- Moore, M.R., Brito, J.A., Qiu, S., Roberts, C.G. & Combee, L.A. 2020. First report of *Meloidogyne enterolobii* infecting Japanese blue berry tree (*Elaeocarpus dicipiens*) in Florida, USA. *Nematology*, 52:1-3. DOI:10.21307/jofnem-2020-005.
- Murithi, H.M., Beed, F., Tukamuhabwa, P., Thomma, B.P.H.J. & Joosten, M.H.A.J. 2016. Soybean production in eastern and southern Africa and threat of yield loss due to soybean rust caused by *Phakopsora pachyrhizi*. *Plant pathology*, 65(2):176-188.

- Musapa M., Kumwenda T., Mkulama M., Chishimba S., Norris D.E., Thuma, P.E. & Mharakurwa, S. 2013. A simple Chelex protocol for DNA extraction from *Anopheles* spp. *Journal of Visualized Experiments*, 71:3281. <https://dx.doi.org/10.3791%2F3281>.
- Neher, D.A. 2001. Role of nematodes in soil health and their use as indicators. *Journal of Nematology*, 33(4):161.
- Newman, M.M., Hoilett, N., Lorenz, N., Dick, R.P., Liles, M.R., Ramsier, C. & Kloepper, J.W. 2016. Glyphosate effects on soil rhizosphere-associated bacterial communities. *Science of the Total Environment*, 543:155-160.
- Ngobeni, G.L., Fourie, H., Donald, A.M. & Mashela, P.W. 2011. Host suitability of selected South African maize genotypes to the root-knot nematode species *Meloidogyne incognita* race 2 and *Meloidogyne javanica*: a preliminary study. *South African Journal of Plant and Soil*, 28(1):49-54.
- Nicol, J.M. & Rivoal, R. 2008. Global knowledge and its application for the integrated control and management of nematodes on wheat. In: Ciancio, A. and Mukerji, K.G., (Eds). *Integrated management and biocontrol of vegetable and grain crops nematodes*. The Netherlands, Dordrecht, Springer. pp. 251–294.
- Ntidi, K.N. 2016. Association and impact of nematodes on weeds and leafy vegetables: with reference to *Meloidogyne*. Potchefstroom: North-West University (PhD Thesis).
- Ntidi, K.N., Fourie, H. & Daneel, M. 2015. Greenhouse and field evaluations of commonly occurring weed species for their host suitability to *Meloidogyne* species. *International Journal of Pest Management*, 62(1):11-19.
- Ntidi, K.N., Fourie, H., Mc Donald, A.H., De Waele, D. & Mienie, C.M. 2012. Plant-parasitic nematodes associated with weeds in subsistence agriculture in South Africa. *Nematology*, 14(7):875-887.
- Odum, E.P. 1985. Trends expected in stressed ecosystems. *Bioscience*, 35(7):419-422.
- Ohnesorg, W.J. & Hunt, T.E. 2015. Managing Soybean Defoliators. Available online: <http://extensionpublications.unl.edu/assets/pdf/q2259.pdf>. Date of access: 27 Jan. 2020.
- Olowe, T. 2011. Relationship between inoculum density levels of *Pratylenchus scribneri*, and growth and yield of maize, *Zea mays*. *International Journal of Nematology*, 21:73-78.
- Omulo, G., Birner, R., Köller, K., Simunji, S. & Daum, T. 2022. Comparison of mechanized conservation agriculture and conventional tillage in Zambia: A short-term agronomic and economic analysis. *Soil and Tillage Research*, 221:105414.
- Onkendi, E.M. 2012. Molecular characterization of root-knot nematodes (*Meloidogyne* spp.) parasitizing potatoes (*Solanum tuberosum*) in South Africa. University of Pretoria (PhD Thesis).

- Onkendi, E.M. & Moleleki L.N. 2013a. Distribution and genetic diversity of root-knot nematodes (*Meloidogyne* spp.) in potatoes from South Africa. *Plant Pathology*, 62:1184-1192. <https://doi.org/10.1111/ppa.12035>.
- Onkendi, E.M. & Moleleki, L.N. 2013b. Detection of *Meloidogyne enterolobii* in potatoes in South Africa and phylogenetic analysis based on intergenic region and the mitochondrial DNA sequences. *European Journal of Plant Pathology*, 136:1–5.
- 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 Pathology*, 63(4):727-737.
- Owenya, M.Z., Mariki, W.L., Kienzie, J., Friedrich, T. & Kassam, A. 2011. Conservation agriculture (CA) in Tanzania: the case of the Mwangaza B CA farmer field school (FFS), Rhotia Village, Karatu District, Arusha. *International Journal of Agricultural Sustainability*, 9(1):145-152.
- Palomares-Rius, J. E., Vovlas, N., Troccoli, G., Landa, B. B. & Castillo, P. 2007. A new root-knot nematode parasitizing sea rocket from Spanish Mediterranean coastal dunes: *Meloidogyne dunensis* n. sp. (Nematoda: Meloidogynidae). *Journal of Nematology*, 39:190- 202.
- Parmeela & Johri, B.N. 2004. Phylogenetic analysis of bacterial endophytes showing antagonism against *Rhizoctonia solani*. *Current science*, pp.687-692.
- Perry, R.N. and Moens, M. 2011. Introduction to plant-parasitic nematodes; modes of parasitism. In Jones, J., Gheysen, G. & Fenoll C., (Eds). *Genomics and molecular genetics of plant-nematode interactions*. Dordrecht: Springer. pp.3-20.
- Phophi, M.M., Mafongoya, P. & Lottering, S. 2020. Perceptions of climate change and drivers of insect pest outbreaks in vegetable crops in Limpopo province of South Africa. *Climate* 8(2):27.
- Poland, J. A. & Rife, T. W. 2012. Genotyping-by-sequencing for plant breeding and genetics. *The Plant Genome*, 5:92–102.
- Powers, T., Harris, T., Higgins, R., Mullin, P. & Powers, K. 2018. Discovery and identification of species using COI DNA barcoding. *Journal of Nematology*, 50(3):399-412.
- Powers, T.O. & Harris, T.S. 1993. A polymerase chain reaction method for identification of five major *Meloidogyne* species. *Journal of Nematology*, 25:1–6.
- Pretorius, M. 2018. The abundance, identity and population dynamics of *Meloidogyne* spp. associated with maize in South Africa. Potchefstroom: North-West University (MSc Dissertation).
- Pretorius, M., Steenkamp, S., Fourie, H. & Mc Donald, A.H. 2014. Host status of commercial wheat and sunflower cultivars to *Meloidogyne* spp. *Journal of Nematology*, 46(2):223.
- Qiu, Z.Q., Mo, A.S., He, Q., Wu, H.Y. & Zhou, X.B. 2016. Root-lesion nematodes on maize in Shandong, China. *Journal of General Plant Pathology*, 82(4):224-227.

- Qwabe, L.R. 2023. Root-knot nematode abundance and diversity in grain fields of New-Era producers in South Africa. Potchefstroom: North-West University (MSc Dissertation).
- Rahi, S. 2017. Research design and methods: A systematic review of research paradigms, sampling issues and instruments development. *International Journal of Economics and Management Sciences*, 6(2):1-5.
- Ramusi, M. & Flett, B. 2014. Foliar Diseases of Sunflower: Alternaria Leaf Blight, Brown Rust and Septoria Leaf Spot; ARC-Grain Crops Institute: Potchefstroom, South Africa. Available online: <https://www.grainsa.co.za/foliar-diseases-of-sunflower:-alternaria-leaf-blight,-brown-rust-and-septoria-leaf-spot>. Date of access: 10 Apr. 2019.
- Randig, O., Bongiovanni, M., Carneiro, R.M. & Castagnone-Sereno, P. 2002. Genetic diversity of root-knot nematodes from Brazil and development of SCAR markers specific for the coffee-damaging species. *Genome*, 45(5):862-870.
- Rashidifard, M. 2019. Comparative molecular and morphological identification, and reproduction potential of South African *Meloidogyne* species with emphasis on *Meloidogyne enterolobii*. Potchefstroom: North-West University (PhD Thesis)
- Rashidifard, M., Fourie, H., Véronneau, P.Y., Marais, M., Daneel, M.S. & Mimee, B. 2018. Genetic diversity and phylogeny of South African *Meloidogyne* populations using genotyping by sequencing. *Scientific Reports*, 8(1):13816.
- Rashidifard, M., Marais, M., Daneel, M. S. & Fourie, H. 2019a. Reproductive potential of South African thermophilic *Meloidogyne* populations, with special reference to *Meloidogyne enterolobii*. *Nematology*, 21:913-921. <https://doi.org/10.1163/15685411-00003263>
- Rashidifard, M., Marais, M., Daneel, M.S. & Fourie, H. 2019b. Morphological and morphometrical identification of *Meloidogyne* populations from various crop production areas in South Africa with emphasis on *M. enterolobii*. *Zootaxa*, 4658(2):251-274.
- Rashidifard, M., Marais, M., Daneel, M.S., Mienie, C.M. & Fourie, H. 2019c Molecular characterisation of *Meloidogyne enterolobii* and other *Meloidogyne* spp. from South Africa. *Tropical Plant Pathology*, 44:213–224. <https://doi.org/10.1007/s40858-019-00281-4>.
- Ribeiro-Oliveira, J.P., de Santana, D.G., Pereira, V.J. & dos Santos, C.M. 2018. Data transformation: an underestimated tool by inappropriate use. *Acta Scientiarum. Agronomy*, 40:e35015.
- Riekert, H.F. 1995. A modified sodium hypochlorite technique for the extraction of root-knot nematode eggs and larvae from maize root samples. *African Plant Protection*, 1:41-43.
- Riekert, H.F. 1996. Economic feasibility of nematode control in dryland maize in South Africa. *African Crop Science Journal*, 4(4):477-481.

- Riekert, H.F. & Henshaw, G.E. 1998. Effect of soybean, cowpea and groundnut rotations on root-knot nematode build-up and infestation of dryland maize. *African Crop Science Journal*, 6:377-383.
- Rossouw, J., Van Rensburg, L., Claassens, S. & Jansen van Rensburg, P.J. 2008. Nematodes as indicators of ecosystem development during platinum mine tailings reclamation. *The Environmentalist*, 28(2):99-107.
- Rusinamhodzi, L. 2015. Crop rotations and residue management in conservation agriculture. In: Farooq, M. and Siddique, K.H.M., (Eds). *Conservation agriculture*. Switzerland: Springer. pp. 21-37.
- Rusinamhodzi, L., Corbeels, M., Zingore, S., Nyamangara, J. & Giller, K.E. 2013. Pushing the envelope? Maize production intensification and the role of cattle manure in recovery of degraded soils in smallholder farming areas of Zimbabwe. *Field Crops Research*, 147:40-53.
- Salamun, P., Hanzelova, V., Miklisova, D., Sestanova, O., Findorakova, L. & Kovacik, P. 2017. The effects of vegetation cover on soil nematode communities in various biotopes disturbed by industrial emissions. *Science of The Total Environment*, 592:106-114.
- Salamun, P., Kucanova, E., Brazova, T., Miklisova, D., Renco, M. & Hanzelova, V. 2014. Diversity and food web structure of nematode communities under high soil salinity and alkaline pH. *Ecotoxicology*, 23(8):1367-1376.
- Sanaullah, M., Usman, M., Wakeel, A., Cheema, S.A., Ashraf, I. & Farooq, M. 2020. Terrestrial ecosystem functioning affected by agricultural management systems: a review. *Soil and Tillage Research*, 196:104464.
- Sánchez-Moreno, S. & Ferris, H. 2018. Nematode ecology and soil health. In Sikora, R. A., Coyne, D., Hallmann, J. and Timper, P., (Eds). *Plant-Parasitic Nematodes in Subtropical and Tropical Agriculture*. Wallingford: UK. CAB International Publishing. pp.62-83.
- Sánchez-Moreno, S., Cano, M., López-Pérez, A. & Rey Benayas, J.M. 2018. Microfaunal soil food webs in Mediterranean semi-arid agroecosystems. Does organic management improve soil health? *Applied Soil Ecology*, 125:138-147.
- Sánchez-Moreno, S., Minoshima, H., Ferris, H. & Jackson, L.E. 2006. Linking soil properties and nematode community composition: effects of soil management on soil food webs. *Nematology*, 8(5):703-715.
- Schmidt, M.A., Hymowitz, T. & Herman, E.M. 2015. Breeding and characterization of soybean triple null; a stack of recessive alleles of Kunitz trypsin inhibitor, soybean agglutinin, and P34 allergen nulls. *Plant Breeding*, 134(3):310-315.
- Schoeman, A. & Craven, M. 2013. New Prospects for Tackling the Problem of Soil-Borne Diseases of Maize. Available online: <https://www.grainsa.co.za>. Date of access: 02 Jun. 2022.

- Schreefel, L., Schulte, R.P.O., De Boer, I.J.M., Schrijver, A.P. & Van Zanten, H.H.E. 2020. Regenerative agriculture—the soil is the base. *Global Food Security*, 26:100404.
- Shurtleff, M. C. & Averre, C.W. 2000. Diagnosing plant disease caused by plant parasitic nematodes. St Paul, Minnesota, USA: The American Phytopathological Society. pp. 187.
- Sibiya, J., Tongoona, P. & Derera, J. 2013. Combining ability and GGE biplot analyses for resistance to northern leaf blight in tropical and subtropical elite maize inbred lines. *Euphytica*, 191(2):245-257.
- Sieriebriennikov, B., Ferris, H. & de Goede, R.G. 2014. NINJA: An automated calculation system for nematode-based biological monitoring. *European Journal of Soil Biology*, 61:90-93.
- Sihlobo, W. 2018. History of Maize Production in South Africa. Available online: <https://wandilesihlobo.com/2018/03/14/history-of-maize-production-in-south-africa-1-min-read/>. Date of access: 04 Feb. 2023.
- Sihlobo, W. 2021. History of Maize Production in South Africa. Available online: <https://wandilesihlobo.com/?s=maize+production+2020%2F2021>. Access 04 Feb. 2023.
- Sikora, R.A., Bridge, J. & Starr, J.L. 2005. Management practices: and overview of integrated nematode management technologies. In: Luc, M., Sikora R.A. and Bridge J., (Eds). *Plant Parasitic Nematodes in Subtropical and Tropical Agriculture*. Wallingford, UK: CAB International Publishing. pp. 793-825.
- Sikora, R.A., Claudius-Cole, B. & Sikora, E.J. 2018. Nematode Parasites of Food Legumes. In: Sikora, R.A., Coyne, D., Hallman, J. and Timper, P., (Eds). *Plant Parasitic Nematodes in Subtropical and Tropical Agriculture*. Wallingford, UK: CAB International Publishing. pp. 290-345.
- Sirias, H.C.I. 2011. Root-knot Nematodes and Coffee in Nicaragua: Management Systems, Species Identification and Genetic Diversity. Swedish University of Agricultural Sciences, Uppsala, Sweden. (PhD Thesis). pp. 57.
- Smiley, R.W. 2015. Root-lesion nematodes: biology and management in Pacific Northwest wheat cropping systems. Pacific Northwest Extension, ed. Oregon State University, Oregon, US. pp. 1-14.
- Smiley, R.W., Gourlie, J.A., Whittaker, R.G., Easley, S.A. & Kidwell, K.K. 2004. Economic impact of Hessian fly (Diptera: Cecidomyiidae) on spring wheat in Oregon and additive yield losses with Fusarium crown rot and lesion nematode. *Journal of Economic Entomology*, 97(2):397-408.
- Smit, E. & Van den Berg, J. 2003. Effect of Tillage Methods On Termite Damage To Maize in South Africa. *International Journal of Tropical Insect Science*, 23(2):153-156.
- Smit, H.A., Tolmay, V.L., Barnard, A., Jordaan, J.P., Koekemoer, F.P., Otto, W.M., Pretorius, Z.A., Purchase, J.L. & Tolmay, J.P.C. 2010. An overview of the context and scope of wheat (*Triticum*

aestivum L.) research in South Africa from 1983 to 2008. *South African Journal of Plant and Soil*, 27(1):81-96.

Smith, C.W. 1995. Crop Production: Evolution, history, and technology. New York. John Wiley and Sons. pp. 57-125.

Sochová, I., Hofman, J. & Holoubek, I. 2006. Using nematodes in soil ecotoxicology. *Environment International*, 32(3):374-383.

Soto, R.L., Padilla, M.C. & de Vente, J. 2020. Participatory selection of soil quality indicators for monitoring the impacts of regenerative agriculture on ecosystem services. *Ecosystem Services*, 45:101157.

South African Agricultural Research Council (ARC). Annual report. Pretoria: ARC; 2018. Available online: <https://www.arc.agric.za/Documents/Annual%20Reports/ARCAAnnualReport-OCTOBER2019-low%20res.pdf> Date of access: 03 Jun. 2021.

South African Bureau for Food and Agricultural Policy (BFAP). 2015. The South African sunflower complex: A report by the Bureau for Food and Agricultural Policy (BFAP). Compiled for the Oilseed and Protein Advisory Development Trust and Oilseed Advisory Committee (OAC). Pretoria: BFAP. Available online: <https://www.bfap.co.za/the-southafricansunflower-complex/> Date of access: 09 Feb. 2021.

South African Bureau for Food and Agricultural Policy (BFAP). 2021. The South African sunflower complex: A report by the Bureau for Food and Agricultural Policy (BFAP). Compiled for the Oilseed and Protein Advisory Development Trust and Oilseed Advisory Committee (OAC). Pretoria: BFAP. Available online: <https://www.bfap.co.za/wp-content/uploads/2023/04/BFAP-Baseline-2021.pdf> Date of access: 09 Feb. 2021.

South African Grain Information Service (SAGIS). 2021. CEC crop estimates [homepage on the Internet]. c2021. Available online: https://www.sagis.org.za/cec_reports_2021.html. Date of access: 14 Mar. 2021.

South African Grain Information Service (SAGIS). 2021. CEC_Report_2021. Available online: https://www.sagis.org.za/cec_reports_2021.html. Date of access, 03 Mar. 2022.

Stapleton, J.J., Summers, C.G., Mitchell, J.P. & Prather, T.S. 2010. Deleterious activity of cultivated grasses (Poaceae) and residues on soilborne fungal, nematode and weed pests. *Phytoparasitica*, 38:61-69.

Steenkamp, S., Fourie, H. & Pretorius, M. 2015. Resistance in sunflower to South African root-knot nematode species. Proceedings of the XVIII International Plant Protection Congress, 24 – 27 August, Berlin, Germany. pp. 237.

Suazo, A. & Hall, H. G. 1999. Modification of the AFLP protocol applied to honeybee (*Apis mellifera* L.) DNA. *Biotechniques*, 26(4):704–705.

- Subbotin, S. A., Ragsdale, E. J., Mullens, T., Roberts, P. A., Mundo-Ocampo, M. & Baldwin, J. G. 2008. A phylogenetic framework for root lesion nematodes of the genus *Pratylenchus* (Nematoda): evidence from 18S and D2–D3 expansion segments of 28S ribosomal RNA genes and morphological characters. *Molecular Phylogenetics and Evolution*, 48:491-505.
- Subbotin, S., Waeyenberge, L. & Moens, M. 2000. Identification of cyst forming nematodes of the genus *Heterodera* (Nematoda: Heteroderidae) based on the ribosomal DNA-RFLP. *Nematology*, 2(2):153-164.
- Subbotin, S.A. 2019. Recombinase polymerase amplification assay for rapid detection of the root-knot nematode *Meloidogyne enterolobii*. *Nematology*, 21:243-251. DOI: 10.1163/15685411-00003210.
- Subbotin, S.A., Sturhan, D., Chizhov, V.N., Vovlas, N. & Baldwin, J.G. 2006. Phylogenetic analysis of Tylenchida Thorne, 1949 as inferred from D2 and D3 expansion fragments of the 28S rRNA gene sequences. *Nematology*, 8:455–474.
- Swanepoel, C.M., Swanepoel, L.H. & Smith, H.J. 2018. A review of conservation agriculture research in South Africa. *South African Journal of Plant and Soil*, 35(4):297-306.
- Tahat, M.M., Kholoud, M.A., Yahia, A.O. & Daniel, I.L. 2020. Soil health and sustainable agriculture. *Sustainability*, 12(12):4859.
- Tan, X., Chang, S.X. & Kabzems, R. 2005. Effects of soil compaction and forest floor removal on soil microbial properties and N transformations in a boreal forest long-term soil productivity study. *Forest Ecology and Management*, 217(2-3):158-170.
- Taylor, A.L. & Sasser, J.N. 1978. Biology, identification and control of root-knot nematodes (*Meloidogyne* species). Raleigh: North Carolina State University, Department of Plant Pathology.
- Tewoldemedhin, Y.T., Lamprecht, S.C., Vaughan, M.M., Doehring, G. & O'Donnell, K. 2017. Soybean SDS in South Africa is Caused by *Fusarium brasiliense* and a novel undescribed *Fusarium* sp. *Plant Disease*, 101: 150–157.
- Thakur, M.P., Del Real, I.M., Cesarz, S., Steinauer, K., Reich, P.B., Hobbie, S., Ciobanu, M., Rich, R., Worm, K. & Eisenhauer, N. 2019. Soil microbial, nematode, and enzymatic responses to elevated CO₂, N fertilization, warming, and reduced precipitation. *Soil Biology and Biochemistry*, 135:184-193.
- Thierfelder, C., Baudron, F., Setimela, P., Nyagumbo, I., Mupangwa, W., Mhlanga, B., Lee, N. & Gérard, B. 2018. Complementary practices supporting conservation agriculture in southern Africa. A review. *Agronomy for Sustainable Development*, 38(2):1-22.
- Thompson, J.P. 2008. Resistance to root-lesion nematodes (*Pratylenchus thornei* and *P. neglectus*) in synthetic hexaploid wheats and their durum and *Aegilops tauschii* parents. *Australian Journal of Agricultural Research*, 59(5):432-446.

- Tigano M, De Siqueira K, Castagnone-Sereno P, Mulet K, Queiroz P, Dos Santos M, Teixeira, C., Almeida, M., Silva, J. & Carneiro, R. 2010. Genetic diversity of the root-knot nematode *Meloidogyne enterolobii* and development of a SCAR marker for this guava-damaging species. *Plant Pathology*, 59:1054–1061.
- Van Bezooijen, J. 2006. *Methods and techniques for nematology*. Wageningen: Wageningen University. pp. 20.
- Van Biljon, E.R. 2004. Crop rotation as part of an integrated pest management programme for the control of plant parasitic nematodes. Proceedings of the Soilborne Plant Diseases Interest Group Symposium, Stellenbosch, 14-16 September 2004.
- Van Biljon, E.R. & Meyer, A. 2000. Reproduction of *Pratylenchus zeae* and *P. delattrei* on crops resistant to *Meloidogyne javanica* and *M. incognita* races 2 and 4. *African Plant Protection*, 6(2):43-45.
- Van den Berg, E., Marais, M. & Swart, A. 2017. Nematode morphology and classification. In: Fourie, H., Spaull, V.W., Jones, R.K., Daneel, M.S. and De Waele, D., (Eds). *Nematology in South Africa: A view from the 21st Century*. Cham, Switzerland: Springer International Publishing. pp. 33–71.
- Van der Laan, M., Bristow, K.L., Stirzaker, R.J. & Annandale, J.G. 2017. Towards ecologically sustainable crop production: a South African perspective. *Agriculture, Ecosystems and Environment*, 236:108-119.
- Van Es, H.M. & Karlen, D.L. 2019. Reanalysis validates soil health indicator sensitivity and correlation with long-term crop yields. *Soil Science Society of America Journal*, 83(3):721-732.
- Van Rensburg, J.B.J. 1988. Efficacy of cloethocarb for control of *Busseola fusca*, *Cicadulina* spp. and nematodes in maize. *Applied Plant Sciences*, 2:63–67
- Vanstone, V.A., Rathjen, A.J., Ware, A.H. & Wheeler, R.D. 1998. Relationship between root lesion nematodes (*Pratylenchus neglectus* and *P. thornei*) and performance of wheat varieties. *Australian Journal of Experimental Agriculture*, 38(2):181-188.
- Varsani, A., Shepherd, D.N., Monjane, A.L., Owor, B.E., Erdmann, J.B., Rybicki, E.P., Peterschmitt, M., Briddon, R.W., Markham, P.G., Oluwafemi, S. & Windram, O.P. 2008. Recombination decreased host specificity and increased mobility may have driven the emergence of maize streak virus as an agricultural pathogen. *The Journal of general virology*, 89(9):2063.
- Venter, C., De Waele, D. & Van Eeden, C.F., 1992. Plant-parasitic nematodes on field crops in South Africa. 4. Groundnut. *Fundamental and Applied Nematology*, 15:7-14.
- Villar-Luna, E., Gómez-Rodríguez, O., Rojas-Martínez, R.I. & Zavaleta-Mejía, E. 2016. Presence of *Meloidogyne enterolobii* on Jalapeño pepper (*Capsicum annuum* L.) in Sinaloa, Mexico. *Helminthologia*, 53(2):155-160.

- Visagie, M., Mienie, C.M., Marais, M., Daneel, M., Karssen, G. & Fourie, H. 2018. Identification of *Meloidogyne* spp. associated with agri-and horticultural crops in South Africa. *Nematology*, 20(4):397-401. <https://doi.org/10.1163/15685411-00003160>
- Von Maltitz, E.F. 1993. The American bollworm, *Heliothis armigera* (Hiibner) (Lepidoptera: Noctuidae) on sunflower. II. Feeding site preferences of the larvae. *Phytophylactica*, 25(4):249-252.
- Vuong, T.D., Sonah, H., Patil, G., Meinhardt, C., Usovsky, M., Kim, K.S., Belzile, F., Li, Z., Robbins, R., Shannon, J.G. & Nguyen, H.T. 2021. Identification of genomic loci conferring broad-spectrum resistance to multiple nematode species in exotic soybean accession PI 567305. *Theoretical and Applied Genetics*, 134:3379-3395.
- Waceke, J.W., Arim, O.J., Waudo, S.W. & Kimenju, J.W. 2013. Plant-parasitic nematodes of maize (*Zea mays* L.) in low input agriculture in Kenya. *African Crop Science Journal*, 8:443–450.
- Waeyenberge, L., Ryss, A., Moens, M., Pinochet, J. & Vrain, T. 2000. Molecular characterisation of 18 *Pratylenchus* species using rDNA restriction fragment length polymorphism. *Nematology*, 2(2):135-142.
- Waeyenberge, L., Viaene, N. & Moens, M. 2009. Species-specific duplex PCR for the detection of *Pratylenchus penetrans*. *Nematology*, 11(6):847-857.
- Walters, J.P., Archer, D.W., Sassenrath, G.F., Hendrickson, J.R., Hanson, J.D., Halloran, J.M., Vadas, P. & Alarcon, V.J. 2016. Exploring agricultural production systems and their fundamental components with system dynamics modelling. *Ecological Modelling*, 333:51-65.
- Walters, M.C. 1979a. The possible status of parasitic nematodes as limiting factors in maize production in South Africa. Technical Communication No. 142. Department of Agricultural Technical Services, Pretoria. pp 62-68.
- Walters, M.C. 1979b. Present status of knowledge of nematode damage and control in South Africa. Technical Communication No. 152. Department of Agricultural Technical Services, Pretoria. pp 62-68.
- Watson, T.T., Forge, T.A. & Nelson, L.M., 2018. Pseudomonads contribute to regulation of *Pratylenchus penetrans* (Nematoda) populations on apple. *Canadian Journal of Microbiology*, 64(11):775-785.
- Williams, C.E. & Clair, D.A.S. 1993. Phenetic relationships and levels of variability detected by restriction fragment length polymorphism and random amplified polymorphic DNA analysis of cultivated and wild accessions of *Lycopersicon esculentum*. *Genome*, 36(3):619-630.
- Windham, G.L. & Williams, W.P. 1988. Reproduction of *Meloidogyne javanica* on corn hybrids and inbreds. *Journal of Nematology*, 20(2):25.

- 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*, 92:884-892.
- Xiang, M., Xiang, P.A., Liu, X. & Zhang, L. 2010. Effect of environment on the abundance and activity of the nematophagous fungus *Hirsutella minnesotensis* in soil. *FEMS Microbiology Ecology*, 71(3):413-417.
- Xu, Y.Y., Lu, H., Wang, X., Zhang, K.Q. & Li, G.H. 2015. Effect of volatile organic compounds from bacteria on nematodes. *Chemistry and Biodiversity*, 12(9):1415-1421.
- Yan, D., Yan, D., Song, X., Yu, Z., Peng, D., Ting, X. & Weng, B. 2018. Community structure of soil nematodes under different drought conditions. *Geoderma*, 325:110-116.
- Yang, B. & Eisenback, J.D. 1983. *Meloidogyne enterolobii* n. sp. (Meloidogynidae), a root-knot nematode parasitizing pacara earpod tree in China. *Journal of Nematology*, 15(3):381.
- Yang, T., Siddique, K.H. & Liu, K. 2020. Cropping systems in agriculture and their impact on soil health-A review. *Global Ecology and Conservation* 23:e01118.
- Yeates, G.W., Bongers, T., De Goede, R.G.M., Freckman, D.W. & Georgieva, S.S.1993. Feeding habits in soil nematode families and genera-an outline for soil ecologists. *Journal of Nematology*, 25(3):315-331.
- Zhang, X., Guan, P., Wang, Y., Li, Q., Zhang, S., Zhang, Z., Bezemer, T.M. & Liang, W. 2015a. Community composition, diversity and metabolic footprints of soil nematodes in differently-aged temperate forests. *Soil Biology and Biochemistry*, 80:118-126.
- Zhang, Z.-y., Zhang, X.-k., Jhao, J.-s., Zhang, X.-p. & Liang, W.-j. 2015b. Tillage and rotation effects on community composition and metabolic footprints of soil nematodes in a black soil. *European Journal of Soil Biology*, 66:40-48.
- Zhong, S., Zeng, H.C. & Jin, Z.Q. 2017. Influences of different tillage and residue management systems on soil nematode community composition and diversity in the tropics. *Soil Biology And Biochemistry*, 107:234-243.
- Zijlstra, C., Donkers-Venne, D.T.H.M. & Fargette, M. 2000. Identification of *Meloidogyne incognita*, *M. javanica* and *M. arenaria* using sequence characterised amplified region (SCAR) based PCR assays. *Journal of Nematology*, 2:847-853.

Annexure 1

Table A4.1a Means and Anova data for Meloidogyne abundance per 50 g grain roots for the 2020 and 2021 surveys conducted on the farmers of 18 grain producers in the Gauteng and Free State provinces of South Africa [*log(x+1) transformed data; **Real means].

Field no and code	Year of survey	Root-knot nematode abundance//50 g roots
22PZ	2021	0*(0**) A
7WM	2021	0,5(3) AB
4CS	2021	0,8(2) ABCDE
4CS_2	2021	0,9(10) ABC
8JB	2021	1,0(4) ABCD
17JB_2	2021	1,1(11) ABCD
22PZ	2020	1,2(191) ABCDE
1SH	2021	2,0(41) ABCDEF
2DM	2021	2,1(16) ABSDEF
17JB_1	2021	2,4(30) ABCDEF
20NR	2021	2,4(25) ABCDEF
17JB_3	2021	2,6(55) ABCDEFG
4CS_2	2020	3,3(107) ABCDEFGH
10MG	2020	3,9(471) ABCDEFGH
16SJ	2021	3,9(242) ABCDEFGH
14DV	2021	4,3(334) ABCDEFGH
10MG	2021	4,3(457) ABCDEFGH
17JB_1	2020	4,7(1783) ABCDEFGH
17JB_3	2020	4,7(234) ABCDEFGH
20NR	2020	4,7(516) ABCDEFGH
18JF	2021	4,8(996) ABCDEFGH
18JF	2020	5,2(539) BCDEFGH
11IM	2021	5,2(239) BCDEFGH
5JM	2020	5,3(388) CDEFGH
17JB_2	2020	5,6(436) CDEFGH
1SH	2020	5,7(940) DEFGH
13PvZ	2021	5,7(929) DEFGH
5JM	2021	5,7(1159) DEFGH
16SJ	2020	5,9(471) EFGH
8JB	2020	6,2(630) FGH
13PvZ	2020	6,3(713) FGH
7WM	2020	6,6(8138) FGH
14DV	2020	6,6(1162) FGH
2DM	2020	7,2(4825) GH
11IM	2020	7,6(2756) H
4CS	2020	7,7(8838) GH
Interaction data: Fields x Surveys		
Fields	P value	0.001
	F ratio	3.75
Surveys (2020 & 2021)	P value	0.001
	F ratio	51.34
Surveys x Field	P value	0.008
	F ratio	1.90

Table A4.1b Means and Anova data for *Rotylenchulus* abundance per 50 g grain roots for the 2020 and 2021 surveys conducted on the farmers of 18 grain producers in the Gauteng and Free State provinces of South Africa [*log(x+1) transformed data; **Real means].

Field no and code	Year of survey	<i>Rotylenchulus</i> abundance/50 g roots
1SH	2020	0*(0**) A
2DM	2020	0(0) A
4CS	2020	0(0) A
20NR	2021	0(0) A
18JF	2021	0(0) A
7WM	2020	0(0) A
17JB_3	2021	0(0) A
17JB_2	2021	0(0) A
11IM	2020	0(0) A
13PvZ	2020	0(0) A
14DV	2020	0(0) A
16SJ	2020	0(0) A
16SJ	2021	0(0) A
17JB_2	2020	0(0) A
17JB_3	2020	0(0) A
18JF	2020	0(0) A
20NR	2020	0(0) A
14DV	2021	0(0) A
1SH	2021	0(0) A
13PvZ	2021	0(0) A
4CS	2021	0(0) A
4CS_2	2021	0(0) A
11IM	2021	0(0) A
8JB	2021	0(0) A
22PZ	2021	0(0) A
5JM	2020	0,6(8) A
4CS_2	2020	0,7(8) A
5JM	2021	1,0(5) AB
17JB_1	2020	1,3(391) ABC
17JB_1	2021	2,1(258) ABCD
7WM	2021	3,5(78) BCDE
10MG	2021	3,8(165) CDE
2DM	2021	3,9(59) CDE
8JB	2020	4,6(141) DE
22PZ	2020	4,7(799) DE
10MG	2020	5,8(412) E
Interaction data: Fields x Surveys		
Fields	P value	0.000
	F ratio	14.60
Surveys (2020 & 2021)	P value	0.273
	F ratio	1.21
		0.000
		9.16
Surveys x Field	P value	
	F ratio	

Table A4.2a Means and Anova data for Meloidogyne abundance per 5 g grain roots for the 2020 and 2021 surveys conducted on the farms of 18 grain producers in the Gauteng and Free State provinces of South Africa [$\log(x+1)$ transformed data; **Real means].

Field no and code	Year of survey	Meloidogyne abundance/5 g roots
4CS_2	2021	0*(114**) A
5JM	2020	0(0) A
8JB	2020	0(0) A
17JB_1	2020	0,5(38) AB
10MG	2020	0,5(155) AB
18JF	2020	0,5(93) ABC
4CS	2021	0,5(317) ABSDEF
17JB_2	2020	0,6(132) ABCD
22PZ	2020	0,7(1) ABCD
17JB_3	2020	0,8(65) ABCDE
2DM	2020	0,9(168) ABCDEF
20NR	2020	1,0(12) ABCDEFG
1SH	2020	1,1(8) ABCDEFG
17JB_2	2021	1,4(250) ABCDEFGH
8JB	2021	1,7(7) ABCDEFGH
7WM	2020	2,0(2) ABCDEFGH
20NR	2021	2,5(3) ABCDEFGH
7WM	2021	2,6(69) ABCDEFGH
5JM	2021	2,8(89) ABCDEFGH
16SJ	2020	2,8(50) ABCDEFGH
4CS_2	2020	2,9(44) ABCDEFGH
2DM	2021	3,0(70) BCDEFGH
13PvZ	2020	3,1(95) BCDEFGH
18JF	2021	3,1(37) BCDEFGH
16SJ	2021	3,1(72) BCDEFGH
10MG	2021	3,2(62) BCDEFGH
17JB_1	2021	3,2(18) BCDEFGH
17JB_3	2021	3,4(119) BCDEFGH
13PvZ	2021	3,4(88) BCDEFGH
14DV	2020	3,5(13) CDEFGH
1SH	2021	3,6(2) DEFGH
11IM	2021	3,8(12) EFGH
14DV	2021	3,9(258) FGH
22PZ	2021	4,0(198) GH
11IM	2020	4,3(5) H
4CS	2020	4,6(51) H
Interaction data: Fields x Surveys		
Fields	P value	0.000
Fields	F ratio	4.83
Surveys (2020 & 2021)	P value	0.000
Surveys (2020 & 2021)	F ratio	32.32
Surveys x Field	P value	0.000
Surveys x Field	F ratio	5.49

Table A4.2b Means and Anova data for Meloidogyne abundance per 5 g grain roots for the 2020 and 2021 surveys conducted on the farms of 18 grain producers in the Gauteng and Free State provinces of South Africa [$\log(x+1)$ transformed data; **Real means].

Field no and code	Year of survey	<i>Pratylenchus</i> abundance/5 g roots
8JB	2020	0*(0**) A
5JM	2020	0(0) A
22PZ	2020	0,4(1) AB
1SH	2020	0,7(8) ABC
20NR	2021	0,7(3) ABC
7WM	2020	0,8(2) ABC
11IM	2020	0,8(5) ABC
1SH	2021	1.0(2) ABCD
8JB	2021	1,3(7) ABCDE
20NR	2020	1,4(12) ABCDEF
14DV	2020	1,6(13) ABCDEF
11IM	2021	2,1(13) ABCDEFG
18JF	2021	2,3(37) ABCDEFG
17JB_1	2021	2,4(38) ABCDEFG
4CS	2021	2,7(317) ABCDEFG
17JB_2	2021	2,9(250) ABCDEFG
17JB_3	2020	3.0(65) ABCDEFG
4CS_2	2020	3,1(44) ABCDEFG
2DM	2021	3,2(70) ABCDEFG
16SJ	2020	3,2(50) ABCDEFG
17JB_1	2020	3,4(38) BCDEFG
7WM	2021	3,4(69) BCDEFG
10MG	2021	3,5(62) BCDEFG
10MG	2020	3,8(155) CDEFG
4CS_2	2021	3,8(114) CDEFG
13PvZ	2020	3,8(95) CDEFG
4CS	2020	3,9(51) BCDEFG
18JF	2020	3,9(93) CDEFG
13PvZ	2021	4,2(88) DEFG
16SJ	2021	4,2(72) DEFG
5JM	2021	4,3(89) DEFG
17JB_3	2021	4,4(119) EFG
2DM	2020	4,6(168) FG
17JB_2	2020	4,7(132) FG
22PZ	2021	5,2(198) G
14DV	2021	5,4(317) G
Interaction data: Fields x Surveys		
Fields	P value	0.000
	F ratio	6.74
Surveys (2020 & 2021)	P value	0.000
	F ratio	13.75
Surveys x Field	P value	0.000
	F ratio	5.34

Table A4.3a Means and Anova data for Dolichodoridae abundance per 200 g soil in grain crop rhizospheres for the 2020 and 2021 surveys conducted on the farms of 18 grain producers in the Gauteng and Free State provinces of South Africa [$\log(x+1)$ transformed data; **Real means].

Field no and code	Year of survey	Dolichodoridae abundance/200 g soil
1SH	2020	0*(0**) A
22PZ	2020	0(0) A
2DM	2020	0(0) A
20NR	2020	0(0) A
18JF	2020	0(0) A
17JB_3	2020	0(0) A
4CS_2	2020	0(0) A
17JB_2	2020	0(0) A
5JM	2020	0(0) A
17JB_1	2021	0(0) A
7WM	2020	0(0) A
7WM	2021	0(0) A
8JB	2020	0(0) A
8JB	2021	0(0) A
10MG	2020	0(0) A
10MG	2021	0(0) A
11IM	2020	0(0) A
17JB_1	2020	0(0) A
13PvZ	2020	0(0) A
13PvZ	2021	0(0) A
14DV	2020	0(0) A
16SJ	2021	0(0) A
16SJ	2020	0(0) A
5JM	2021	0,3(1) A
11IM	2021	0,5(1) AB
4CS_2	2021	0,5(4) AB
22PZ	2021	0,6(2) AB
18JF	2021	0,8(2) AB
4CS	2021	0,9(3) ABC
17JB_2	2021	1,5(6) ABCD
17JB_3	2021	2,3(24) BCD
14DV	2021	2,9(60) CDE
4CS	2020	2,9(50) CDE
2DM	2021	3,1(89) DE
20NR	2021	4,6(200) E
1SH	2021	4,7(266) E
Interaction data: Fields x Surveys		
Fields	P value	0.000
	F ratio	11.17
Surveys (2020 & 2021)	P value	0.000
	F ratio	85.97
Surveys x Field	P value	0.000
	F ratio	11.83

Table A4.3b Means and Anova data for *Pratylenchus* abundance per 200 g soil in grain crop rhizospheres for the 2020 and 2021 surveys conducted on the farms of 18 grain producers in the Gauteng and Free State provinces of South Africa [$\log(x+1)$ transformed data; **Real means].

Field no and code	Year of survey	<i>Pratylenchus</i> abundance/200 g soil
1SH	2020	0*(0**) A
8JB	2020	0(0) A
17JB_2	2021	0,4(1) AB
11IM	2021	0,7(2) ABC
1SH	2021	0,8(2) ABC
5JM	2021	0,9(2) ABC
17JB_1	2020	1.0(6) ABC
4CS_2	2021	1.0(4) ABC
5JM	2020	1.0(4) ABC
4CS	2021	1,2(6) ABCDE
8JB	2021	1,2(4) ABCD
20NR	2021	1,2(13) ABCDE
20NR	2020	1,2(13) ABCDE
2DM	2020	1,2(13) ABCDE
2DM	2021	1,4(6) ABCDE
11IM	2020	1,7(9) ABCDE
14DV	2021	2,1(8) ABCDE
13PvZ	2021	2,3(13) ABCDEF
16SJ	2020	2,3(31) ABCDEF
17JB_1	2021	2,4(6) ABCDEF
17JB_2	2020	2,5(49) ABCDEF
16SJ	2021	2,6(25) ABCDEF
18JF	2021	2,6(49) ABCDEF
18JF	2020	2,6(49) ABCDEF
17JB_3	2021	2,6(16) ABCDEF
10MG	2021	2,6(21) ABCDEF
13PvZ	2020	2,6(18) ABCDEF
14DV	2020	2,9(27) BCDEF
17JB_3	2020	3,3(49) CDEFG
7WM	2021	3,5(35) CDEFG
7WM	2020	4.0(69) CDEFG
4CS_2	2020	4,0(60) EFG
4CS	2020	4,2(83) CDEFG
22PZ	2020	4,6(102) FG
22PZ	2021	4,6(102) FG
10MG	2020	5,9(380) G
Interaction data: Fields x Surveys		
Fields	P value	0.000
	F ratio	10.55
Surveys (2020 & 2021)	P value	0.001
	F ratio	12.25
Surveys x Field	P value	0.000
	F ratio	3.43

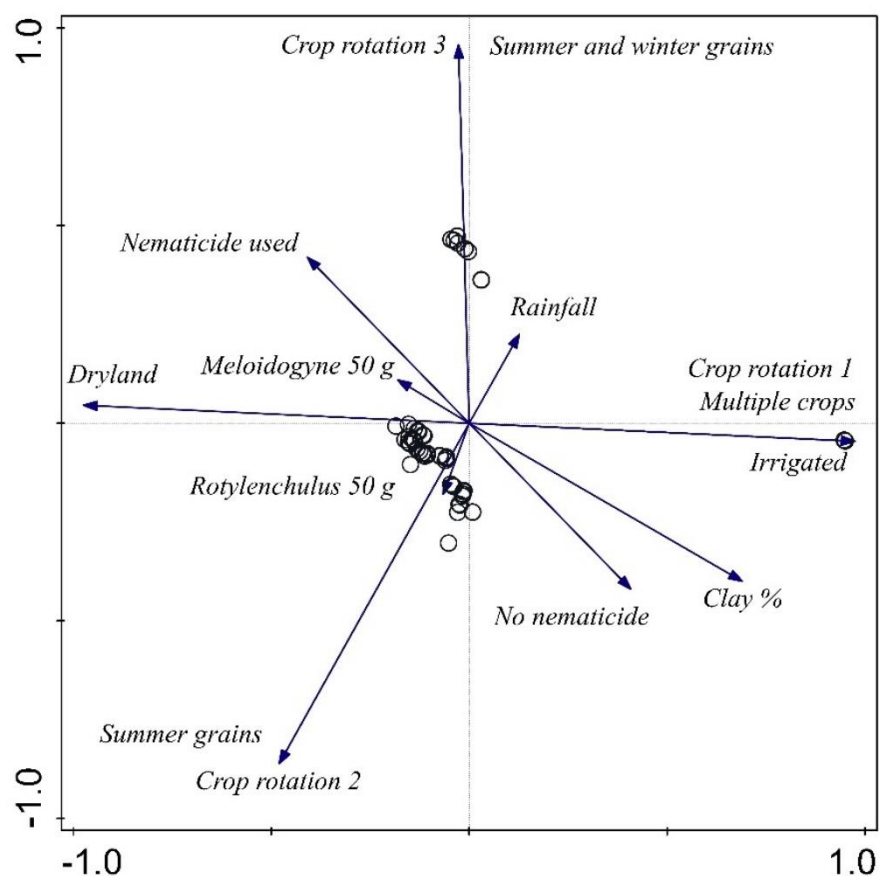


Figure 1: Principal component analysis (PCA) illustrating the relationship between plant-parasitic nematodes (*Meloidogyne* and *Rotylenchulus*), abiotic and other parameters for 50 g roots sampled during survey 1 and 2.

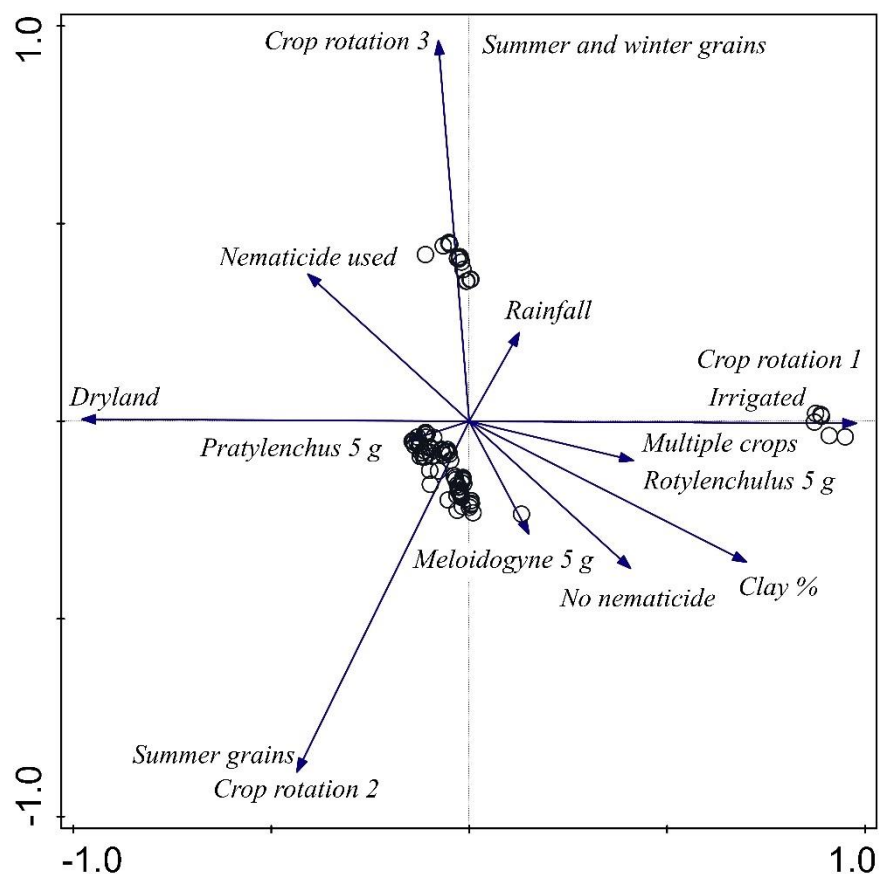


Figure 2: Principal component analysis (PCA) illustrating the relationship between plant-parasitic nematodes (*Meloidogyne*, *Pratylenchus* and *Rotylenchulus*), abiotic and other parameters for 50 g roots sampled during survey 1 and 2.

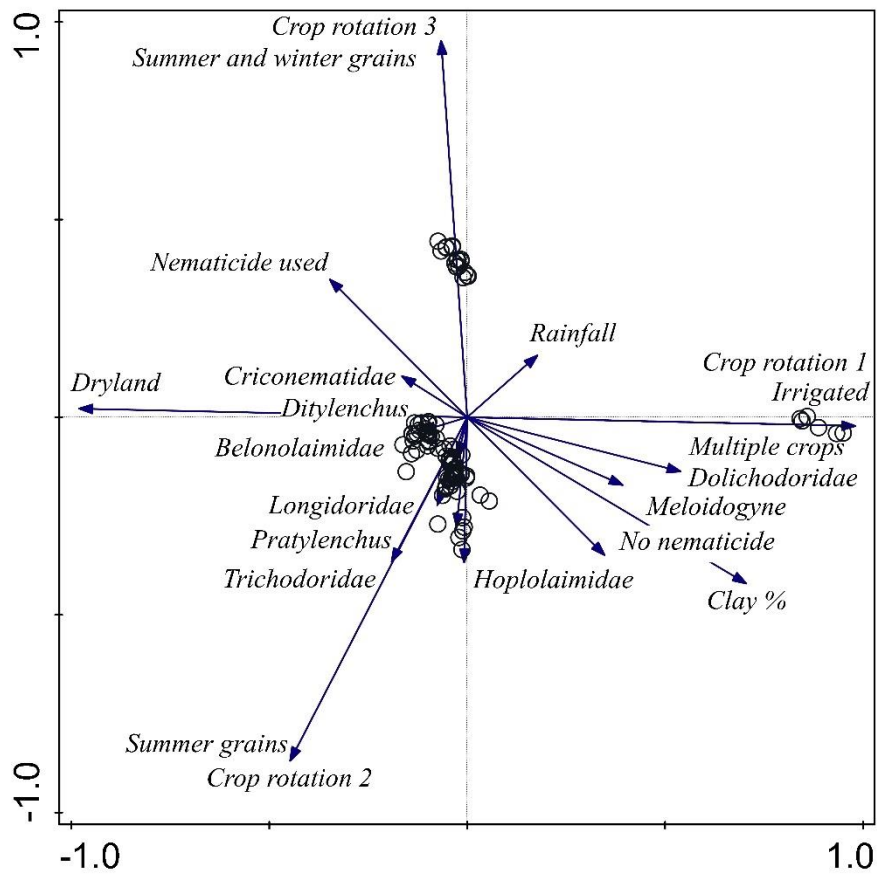


Figure 3: Principal component analysis (PCA) illustrating the relationship between plant-parasitic nematodes (*Meloidogyne*, *Pratylenchus* and *Rotylenchulus*), abiotic and other parameters for 200 g roots sampled during survey 1 and 2.

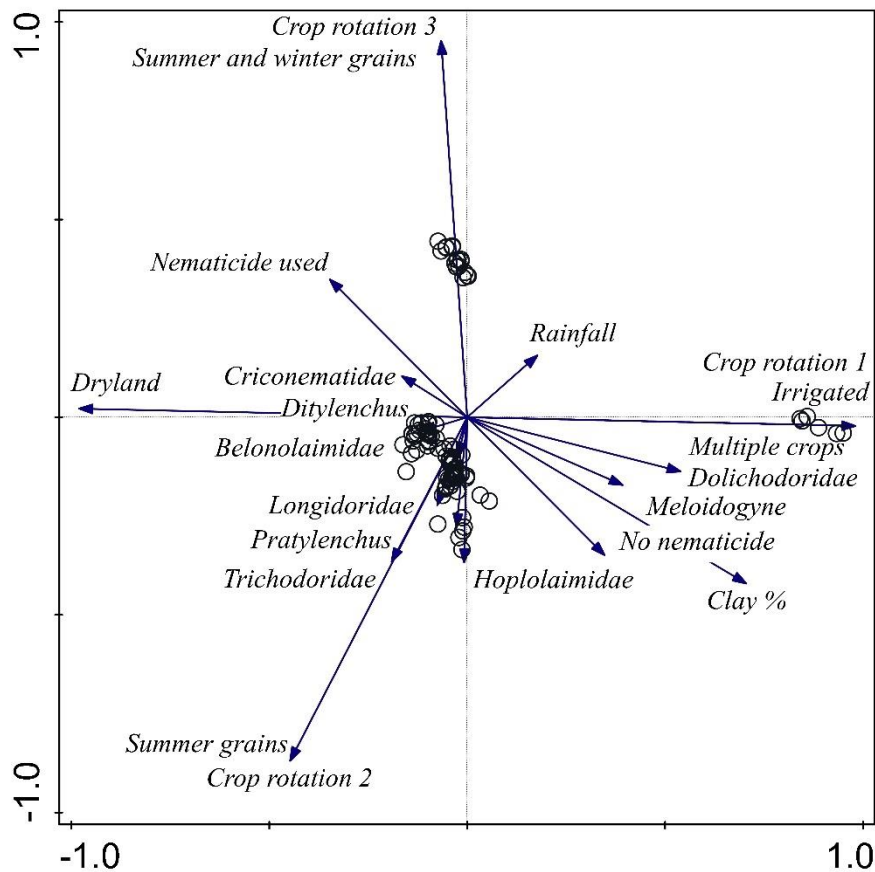


Figure 4: Principal component analysis (PCA) illustrating the relationship between plant-parasitic nematodes, beneficial nematodes, abiotic and other parameters for 200 g soil sampled during survey 2.

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