Root-knot nematode abundance and diversity in grain fields of New-Era producers in South Africa

LR Qwabe

orcid.org/0000-0002-0175-5937

Dissertation accepted in fulfilment of the requirements for the degree *Master of Science in Environmental Sciences with Integrated Pest Management* at the North-West University

Supervisor:	Prof H Fourie
Co-supervisor:	Dr KN Ntidi

Graduation May 2023

35086254

DEDICATION

I would like to dedicate my thesis to my late Uncle, Bersely Madala Gumede, who played a very significant role in my life. I hope this would have made you proud.

We miss you!

ACKNOWLEDGEMENTS

I sincerely thank my heavenly father who has guided me all my life and has given me tremendous courage and strength throughout this period.

Words alone are not enough to express my deepest gratitude to my supervisory team; Prof. Hendrika Fourie and Dr. Nancy Ntidi for their professional guidance, patience and assistance in various aspects of the success of my study. They played a crucial role in my journey to completing my study. I extend a special thanks to my co- supervisor, Dr. Nancy Ntidi for deeming me the honour of working with her on this project.

I would like to thank Bongiwe Mhlongo, Danny-boy Styen, Anè Loggenberg, Raymond Collett, and Dr. Gerhard Engelbrecht not only for their technical assistance with this study, but also for demonstrating how fascinating and dynamic the field of nematology is.

I am grateful to my parents, Mrs Promise Mbatha and Mr Sizwe Mbatha, as well as my friends, for their encouraging words; they have been a pillar of my strength throughout this journey, and I have no idea how I would have made it without them.

I am eternally grateful to the GrainSA and The Sasol Agricultural Trust funders for their financial support.

I would like to thank the maize farmers who contributed to this study by allowing me to take samples from their fields, without them this study would not have been possible.

ABSTRACT

Production of maize, the third most important grain crop for human consumption after rice and wheat, is hampered by *Meloidogyne* which is the economically most important plant-parasitic nematode (PPN) genus infecting and damaging local grain crops. The research aim of this study was to determine the abundance and diversity of PPN in local grain fields of New-Era producers. Root and rhizosphere samples were obtained during the 2018/19 and 2019/20 growing seasons from 15 fields on which maize (2019 & 2020) and sunflower (2020) were grown. Eggs of sessile endoparasitic genera, e.g., *Meloidogyne* and *Rotylenchulus*, were extracted from 50 g roots using an adapted NaOCl method, while motile individuals of these two and other PPN genera were extracted from 5 g roots using the sugar-flotation method. From soil samples, both PPN and beneficial nematodes were extracted using the decanting and sieving method, followed by the sugar-flotation method. Prominence values (PVs) were calculated for all nematode genera and/or families, while univariate and multivariate data analyses were done to elucidate the population dynamics of predominant PPN genera over four sampling intervals and investigate whether associations existed between nematodes and selected abiotic factors. Only *Meloidogyne* and *Rotylenchulus* occurred in 50 g root samples, with *Meloidogyne* being predominant. These two genera also dominated in 5 g roots, with *Helicotylenchus* and *Pratylenchus* also being present but to a lesser extent. In soil, *Rotylenchulus* was predominant, followed by *Helicotylenchus*, *Meloidogyne* and *Pratylenchus* while other genera and families occurred in low densities. Beneficial trophic groups in the soil, in order of domination, were bacterivores, fungivores, omnivores and predators. *Meloidogyne* and *Rotylenchulus* abundance in 50 g roots, and that of fungivores in soil were the highest at Dwaalkraal and Hoopstad for the 2nd sampling intervals of both surveys. For the 2019 survey, localities with soils containing high sand (87 - 93%), K (293 - 240 mg/kg) and N-NH_4 (1.05 - 1.75 mg/kg) strongly correlated with high *Meloidogyne* and *Rotylenchulus* abundance in 50 g roots, and that of bacterivores in soil. For the 2020 survey, high *Meloidogyne* abundance in 50 g roots and high fungivore abundance in soil correlated with localities containing soil with high Sand (84 - 94%) and pH values (5.25 - 6.6). Morphological and molecular species identification for *Meloidogyne* evidenced *M. incognita* as the predominant species, followed by *M. javanica*, *M. arenaria* and *M. enterolobii.* These results revealed information that is novel and crucial for proper planning of grain crop production in fields where mostly communities containing mixed *Meloidogyne* spp. occur. The population densities of *Meloidogyne* and

Rotylenchulus were exceptionally high in root samples, while the latter genus had high densities in soil samples too. This suggests that practicing monoculture as well as rotation of maize and sunflower are favorable to high population density build-ups of these two genera. Despite the known adverse effect of *Meloidogyne* on maize and sunflower, the impact of *Rotylenchulus* on both crops is unknown. Findings of this study will assist nematologists and producers to make informed decisions to combat nematode problems in maize-based cropping systems.

Key words: Maize, *Meloidogyne*, nematodes population/grain, *Rotylenchulus*, sunflower.

TABLE OF CONTENTS

DEDICATION ..... **i**
ACKNOWLEDGEMENTS ..... **ii**
ABSTRACT ..... **iii**
CHAPTER 1: INTRODUCTION AND LITERATURE REVIEW **1**
1.1 General introduction 1
1.1.1 Problem statement and justification 2
1.1.2 Aim and objectives 3
1.1.3 Study outline 4
1.2 Literature review 5
1.2.1 Grain crop production in South Africa 5
1.2.2 *Zea mays* L. 5
1.2.3 Sunflower *Helianthus annuus* L. 10
1.3 Nematodes 13
1.3.1 Classification of plant-parasitic nematodes and basic biology, and morphology 13
1.4 Identification of *Meloidogyne* spp. 19
1.4.1 Morphological and morphometrical identification 19
1.4.2 Molecular and biochemical identification of *Meloidogyne* spp. 24
1.5 Abiotic parameters influencing root-knot nematodes 29
1.5 Management of root-knot nematodes 30
1.5.1 Chemical control 30
1.5.2 Cultural control 31
1.5.4 Integrated nematode management (INM) 34
2. Conclusion 35
CHAPTER 2: MATERIALS AND METHODS **36**
2.1 Survey 36
2.1.1 Soil and root sampling for nematode extractions 42
2.1.2. Roots (5 g and 50 g) and soil (200 g) processing 42
2.2 Counting and identification of nematode genera 45
2.3 Rearing of root-knot nematode species for identification purposes 45
2.3.1 Morphological identification of root-knot nematodes 46
2.4 Biochemical and molecular identification of Meloidogyne spp. 48
2.4.1 Isolation of females 48

3.1 Statistical analyses of nematode data.....51
CHAPTER 3: RESULTS AND DISCUSSION - NEMATODE SURVEY 52
3.1 Results.....52
3.11 Nematode data – 50 g roots.....52
3.2.2 Nematode data 5 g roots.....59
3.2.3 Plant-parasitic nematode genera and beneficial nematodes/200 g soil samples.....67
3.3 Associations between nematodes and selected abiotic parameters.....79
3.4 Discussion 85
CHAPTER 4: RESULTS AND DISCUSSION - MORPHOLOGICAL AND MOLECULAR IDENTIFICATION 91
4.1 Results.....91
4.2.1 Morphological/classical identification.....91
4.2.2 Molecular identification 94
4.3 Discussion 99
CHAPTER 5: CONCLUSION 103
5.1 GENERAL CONCLUSION.....103
5.2 TESTING OF THE HYPOTHESES 103
5.3 FUTURE RECOMMENDATIONS.....105
6. REFERENCES 106
APPENDIX 1 135

CHAPTER 1: INTRODUCTION AND LITERATURE REVIEW

1.1 General introduction

It is projected that the world's population will increase to 8 billion people by November 2022, to 8.5 billion in 2030 and to 9.7 billion in 2050 (UN, 2022). This predicted rapid population growth will worsen food insecurity and human malnutrition in developing countries, especially those located in sub-Saharan Africa (SSA) (Dodo, 2020).

According to the Organization for Economic Co-operation and Development and the Food and Agriculture Organization (OECD-FAO) (2022), major food crops generally consumed by people in developing countries include maize (*Zea mays* L.), wheat (*Triticum aestivum* L.) and rice (*Oryza sativa* L.) in most of the SSA regions, including South Africa (SA). Maize remains the staple food for inhabitants (Awata *et al*., 2019; Ekpa *et al*., 2019). In SA, maize also provides financial benefits to developing producers and small households in rural areas, since it is sold as green mealies as well as dry seed and processing into mealie meal in the townships (Hlongwane *et al*., 2014) and/or delivered to district markets (Hagos & Geta, 2016). By contrast, during 2018 it was estimated that more than 9 000 commercial producers produced the majority of maize in SA, and thousands of small-holder produced the rest (DAFF, 2018). Maize also contributes greatly as a feed source in livestock and poultry industries (Adisa *et al*., 2019). Recently, maize production locally experienced a high adoption rate with increased intensification of cropping systems amongst rural farming communities for semi-commercial and/or commercial purposes. Therefore, this study focused on 'New-Era Commercial' maize producers in SA which is defined as producers who produce commercial harvests of over 250 tons (GrainSA Farmer Development Programme (FDP): Unpublished). The adoption in maize production by developing producers may be accompanied by the crop being exposed to existing, but also new diseases and pests that have the potential to seriously hamper production.

Plant-parasitic nematodes (PPN), particularly root-knot (RKN: *Meloidogyne* Göldi, 1887) and root-lesion nematodes (RLN: *Pratylenchus* Filipjev, 1936) are considered the most damaging genera and pose a threat to local maize producers, both commercial and

developing (Mashela *et al*., 2017; Mc Donald *et al*., 2017; Dhau *et al*., 2018). The potential adverse impact of these genera is especially important keeping in mind the recent progress in and adoption of maize production by the local rural farming communities for semi-commercial and/or commercial purposes. Research to date include two surveys done in gardens and/or small fields of subsistence farmers indicating that *Meloidogyne* and *Pratylenchus* spp. were the most abundant and widely distributed (Mtshali *et al*., 2002a; Daneel *et al*., 2003), while no data have been generated for developing commercial and semi-commercial producers on this topic. As a result, this study was initiated to assist New-Era grain producers with information about nematode pests that can cause problems in their fields. Such a contribution towards the science of nematology and to the daily livelihood of producers will knowledge of these pests to enable producers to have better understanding and to endeavour to sustainably produce of the major grain food and fodder crops.

1.1.1 Problem statement and justification

In South Africa, the 'Massive Food Production Programme (MFPP)' has assisted maize producers that were previously regarded as rural, small-holder producers to shift towards semi-commercialisation and/or commercialisation in terms of production of the crop (Mtero, 2012). Consequently, this intensification in maize production coupled with poor crop husbandry can lead to an increase in problems being experienced resulting from infection by PPN and/or other soil pathogens (Coyne *et al*., 2018). Plant-parasitic nematodes are often overlooked (Talwana *et al*., 2016), but are major threats to producers accounting for global crop losses of approximately to $US 80 billion per year (Nicol *et al*., 2011; Navia *et al*., 2017).

Most producers in developing countries have poor infrastructure resources and limited financial access, and as a result, they are not aware of the severe damage PPN may cause to crop yields (quality and quantity) (Coyne *et al*., 2018). Producers often attribute such damage to other pest and disease infestations and/or other biotic and abiotic crop related problems (Sigh *et al*., 2015). The aerial symptoms induced by nematode parasitic to plants are usually non-specific (Phani *et al*., 2021) and often resembles those caused by other abiotic and/or biotic constraints (Sikora *et al.*, 2005; McSorley 2011; Ntidi *et al.,* 2012).
Hence, it is generally recommended to producers to remove and study the below-ground parts of plants where specific nematode genera cause specific symptoms, e.g., *Meloidogyne*

spp. resulting in galling of roots/other below ground plant parts (Engelbrecht, 2020). However, producers rarely do this, perhaps due to a concern highlighted by Collet (2020), that commercial and subsistence producers have insufficient information about PPN. As a result, they lack awareness of perceiving PPN as damaging pests (Phani *et al*., 2021). Furthermore, producers perpetuate the spread of nematode infestation with farm equipment due to their lack of knowledge and ignorance regarding PPN being present in their crop fields (Lambert & Bekal, 2002). This negligence by subsistence producers has increased the necessity for generating in-depth scientific knowledge and an understanding of nematode pests that can damage maize crops in the developing agricultural sector of SA.

1.1.2 Aim and objectives

1.1.2.1 Aim

The aim of the study was to generate empirically based information on the abundance and diversity of PPN assemblages in grain fields of black New-Era producers, with a particular focus on *Meloidogyne* spp.

1.1.2.2 The objectives of this study were to determine:

The first objective was to determine the abundance, diversity, and occurrence of PPN species in fields of New-Era producers situated in three provinces of South Africa (Free-State, North-West and Gauteng) by means of a baseline study, with focus on *Meloidogyne* spp. In addition, the existence of associations between nematodes and selected abiotic parameters was investigated.

The second objective was to identity *Meloidogyne* spp. that were present in the fields sampled, using morphological and molecular methods.

1.1.2.3 The hypotheses of the study

The two hypotheses derived from the objectives represent the following:

i) that the predominant PPN genus in fields of the New-Era producers is foreseen to be root-knot nematodes (RKN), while it is also expected that associations between nematodes (PPN and beneficial) and some of the selected abiotic parameters will be evidenced and

ii) that the most commonly occurring and predominant RKN species expected to be identified from the 15 fields of farmers are *Meloidogyne incognita* and *M. javanica*.

1.1.3 Study outline

This study was structured to contain five chapters, namely:

- Chapter 1 highlights some aspects of the maize and sunflower (*Helianthus annuus* L.) crops, e.g., origin, morphology, production practices and its importance, followed by a section reviewing various aspects of PPN with focus on the two known economically important genera, *Meloidogyne* and *Pratylenchus* spp., alluding to its morphology, physiology, distribution, and management. It also covers information about various identification techniques (morphology, morphometrics and molecular) for the diagnosis of *Meloidogyne* spp., which is hypothesized to be the predominant genus that will be recorded in the fields sampled.
- Chapter 2 includes detailed information about the methodologies applied to conduct all the experimental activities during this research project such as those related to the survey, nematode extraction and counting, and identification of *Meloidogyne* spp. using morphological and molecular techniques.
- Chapter 3 focuses on the data (abundance, diversity, and occurrence) generated for PPN and trophic groups of beneficial nematodes. In addition, analyses of selected abiotic parameters are investigated in terms of their association with nematode assemblages.
- Chapter 4 contains the outcomes of the morphological (focusing on perineal-patterns of females in particular) and molecular identification of *Meloidogyne* spp. using the sequence characterised amplified regions-polymerase chain reaction (SCAR-PCR) technique.
- Chapter 5 is dedicated to conclusive remarks and future recommendations about the survey results and the identification of *Meloidogyne* spp. from this study, with reference as to how producers can use it to assist them in producing crops sustainably in the presence of *Meloidogyne* and other damaging nematode genera and families to ensure food security.

1.2 Literature review

1.2.1 Grain crop production in South Africa

In South Africa (SA), the main crops cultivated in rotation with maize (*Zea mays* L.) are sunflower (*Helianthus annus* L.), soybean (*Glycine max* L. Merr.), and groundnut (*Arachis hypogaea* L.) (Steenkamp, 2012) (Table 1.1). However, maize mono-cropping is the predominant practice in most maize fields in SA (Haarhoff *et al*., 2020). Furthermore, most semi-commercial producers where samples were obtained for this study grow maize in monoculture, with only a few rotating it with sunflower (Personal communication: Dr Nancy Ntidi, ARC-Grain Crops, Potchefstroom, September 2022) (see Chapter 2, table 2.1). As a result, the focus will be on maize, which is the major grain crop produced in SA, and sunflower production in terms of an introduction and overview to this study (Tables 1.3 & 1.4).

Table 1.1 Production figures for maize and other grain crops grown in South Africa during the summer-growing season of 2021/22 (USDA, 2022).

Crop	**Planted (Million ha)**	**Production (Million metric tons)**
Maize (*Zea mays* L.)	5.67	17.00
Sunflower (*Helianthus annuus* L.)	1.55	0.90
Soybean (*Glycine max*)	2.00	1.85
Sorghum (*Sorghum bicolor*)	3.78	0.17
Groundnut (*Arachis hypogaea* L.)	1.86	0.07

1.2.2 *Zea mays* L.

1.2.2.1. Production figures

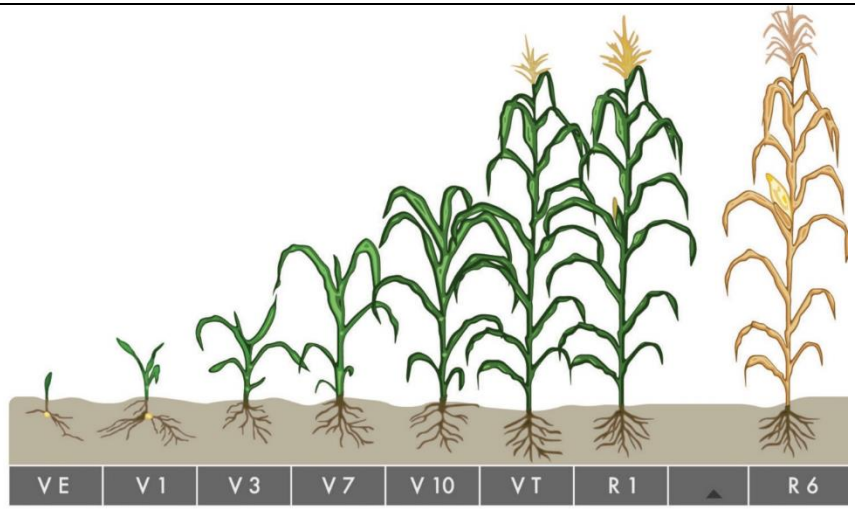
World maize production exceeded 1205.4 million metric tons (MMT) during the 2021-2022 production season with top five world countries producing it being the United States of America (USA), China, Brazil, Argentina, and Ukraine (Table 1.2). South Africa ranks 9th with 17 MMT and is the major maize producer on the African continent (USDA, 2022).

Table 1.2 Maize production figures in selected world countries for the 2021-2022 growing season (USDA, 2022)

Country	**Area on which maize is produced (Million hectare)**	**Yield of maize produced (Metric tons per hectare)**	**Production of maize (Million metric tons)**
Globally (except Africa)			
United States of America	34.5	11.1	383.9
China	43.3	6.3	272.6
Brazil	20.8	5.5	114
Argentina	6.8	7.9	54
Ukraine	5.3	7.9	42
Africa			
South Africa	3	5.7	17
Nigeria	6	1.9	11.6
Ethiopia	2.4	3.8	9

A concise overview of information about maize and sunflower and the requirements for production of the crops are given in Tables 1.3 and 1.4.

Table 1.3 A summary of basic information on maize and production requirements to optimize maize yield under South African climatic conditions.

Origin and distribution	Maize cultivation started in the Americas approximately 7 000 – 10 000 years ago (Piperno & Flannery, 2001; Hallauer *et al*., 2010) being introduced in the 15th century (Farnham *et al*., 2003). Production of the crop spread to tropical Africa in the 16th century (Miracle, 1965; Smale & Jayne, 2003), to South Africa in the mid-17th century (Mc Cann, 2001), and then to the rest of the world (AGOGTR, 2008; Da Fonseca, 2015).
Taxonomic classification	Taxonomy classification according to Awata *et al*. (2019) Kingdom Plantae Class Liliopsida (Monocotyledon) Subclass Commelinidae Order Cyperales Family Poaceae (Grass family) Subfamily Panicoideae Genus *Zea* Species *mays*
Growth stages	 **Fig. 1.1** A schematic illustration of different vegetative (Ve? to V9) and reproductive (R1 to R6) stages of maize linked to its growth and development (Illustration: https://www.pioneer.com/us/agronomy/staging_com_growth.html).
Practices used for growing maize	Maize production is done under practices representing conventional- (CT), conservation- (CA) and regenerative agriculture (RA). Although CA practice adoption increased globally from 45 million hectares (ha) in 1999 to 95 million ha in 2005, it has been estimated to reach approximately 180 million hectares (Mha) in 2020 (Somasundaram *et al*., 2020). Despite the benefits, Africa has limited CA adoption, particularly among smallholders (Brown *et al*., 2017). According to Haarhoff *et al*. (2020), most maize fields in SA are still managed with rigorous soil tillage and continuous maize monoculture. Conventional agriculture, relies heavily on external inputs,e.g., pesticides, herbicides, and inorganic fertilizers, for crop production (De Ponti *et al*. (2012), and contributes to soil erosion as well as pollution of natural resources (air, soil and water). By contrast, CA is defined by the

	FAO (2018a) as farming systems that conform to the standards of no or minimal till practices and feasible farming techniques *viz;* soil cover and crop diversity through crop rotation to maintain and improve soil quality. It increases the population densities and diversity of beneficial soilborne organisms, replenishing nutrients depleted by long-term tillage and poor soil management (FAO, 2008). However, no-tillage contributes to poor crop establishment, subsoil compaction, and high grain yield variability (Haarhoff *et al*., 2020). Regenerative agriculture is also gaining popularity in SA and is defined by Schreefel *et al*. (2020) as an approach to farming that uses soil conservation as the entry point to regenerate and contribute to multiple ecosystem services with animal grazing and the all-year-round presence of crop/plant roots in the soil supplementing the three principles of CA, namely no-or minimum tillage, soil cover and crop rotation (FAO, 2018a).
Factors influencing maize production	
Climatic requirement	Maize is typically grown in areas with ≥19 °C daily average temperatures (DAFF, 2007). The optimal temperature for seed germination is between 16 -18 °C and flowering occurs best between 19-25 °C (DAFF, 2008). In SA, 91 % of maize is produced under rainfed conditions (Haarhoff, 2020). Annual rainfall of 500 - 750 mm is required for optimal yield and quality (DAFF, 2008). However, annual rainfall ranging from 350 - 450 is ideal to obtain a yield of 3152 kg ha^{-1} (DAFF, 2008).
Soil types	Maize grows well on several soil types including loamy soils, but the crop thrives best in soils containing 10-30% clay with an effective depth being ≥ 900 mm. In addition, adequate and stabilized amounts of plant nutrients, and a pH (H_2O) of above 5.8 are important to maize production (DAFF, 2008).
Fertilization	The amount of fertilizer applied is determined by the soil fertility status, season, and cultivar (DAFF, 2008). Nitrogen (N), phosphorous (P), and potassium (K) are the three main elements used by farmers in fertilizer mixtures, with N and K applications not to be exceeding 70 kg/ha, 50 kg/ha, and 30 kg/ha for 0.9 m, 1.5 m, and 2.1 m row widths, respectively. Larger amounts can be applied if placed 70 -100 mm next to and below the seeds (DAFF, 2008).
Cultivar choice	Variety selection is achieved by considering yield potential, stability of yield, standability, compensation ability, growing season length, germination and vigor, disease, insect tolerance and weed control (GrainSA, 2021a). Soil potential and the producer's management potential will always play a big role in the selection of cultivars. Most local maize producers prefer cultivars that mature early, have high yield potentials and prolificacy, are resistant to economically important diseases, display white grain colour, and have superior drying and shelling characteristics (Sibiya *et al*., 2013). In addition, maize producers plant maize cultivars such as PAN2432R, PAN5R691BR, PANSA154ZMK and Pioneer1745 and others (Table 2.1 a & b).
Planting time and depth	The planting time of maize is determined by the rainfall patterns and temperature, and the length of the growing season. Maize is generally planted during late spring/late summer, with ideal planting periods in November and December (DAFF, 2020). The planting depth of maize seeds is influenced by soil type and planting date, but varies from 5 - 10 cm (DAFF, 2008). Maize seeds are planted at a different spacings, inter- and intrarow, from region to region, depending on the agroclimatic conditions, the cropping system (monoculture or rotation) and the hybrid (Ngoune Tandzi & Mutengwa, 2020).
Harvesting	Harvestable maturity is generally indicated by the appearance of a black layer at the tip of the kernel (detachment of the kernel from the cob) (DAFF, 2008). Small maize fields are typically harvested by hand by developing producers (Du Plessis, 2003). Commercial producers harvest maize seeds mechanically and dry them in the sun or in commercial dryers, with the optimum equilibrium moisture content ranging from 12 - 14% for both long-term and short-term storage (Sadaka & Bautista, 2012; Kenneth & Hellevang, 2013).
Disease and weed control	Pest and disease control using resistant cultivars, such as those containing the *Bacillus thurigiensis* (Bt) genes (single or stacked) effective against African maize stem/stalk borers (*Busseola fusca*, Fuller, 1901), is popular and used widely by local farmers. The same applies to weed control using hybrids with the Roundup Ready®-tolerant (RR) gene(s). Various hybrids contain both the RR and Bt genes (Nelson, 2001). Other diseases such as spot, rust (*Puccinia recondite*) and tassel smut (*Sphacelotheca reiliana*), stem rot (*Sclerotinia sclerotiorum*) and root rots (*Phytophthora*

	megasperma are controlled based on planting disease free seeds, soil cultivation, weed control and crop rotation (DAFF, 2003). Maize pests including maize snout beetle *(Sitophilus zeamais)*, black maize beetle *(Heteronychus arator* and armyworm (*Spodoptera frugiperda*), to mention a few only, are mainly controlled by chemicals, soil cultivation and planting date (DAFF, 2003). In terms of plant-parasitic nematodes (PPN), several local maize hybrids and open-pollinated hybrids with resistance to the commonly occurring *Meloidogyne* spp. have been identified, namely *Meloidogyne javanica* (Treub, 1885), Chitwood, 1949, and *Meloidogyne incognita* (Kofoid and White, 1919) that infect and damage the crop globally (Windham and Williams, 1994; Adegbite, 2011; Ngobeni *et al*., 2010; Kagoda *et al*., 2011, 2015; Dababat & Fourie, 2018).
PPN interactions with other pathogens	Bacteria, fungi, and viruses occur in root tissue where *Meloidogyne* spp. are found and form complexes. Typically, damaged root tissues of maize plants attributed to *Meloidogyne* spp. infection has been found to be vulnerable to most bacteria including *Pseudomonas* spp. *Agrobacterium* spp. (Taylor & Sasser, 1978). Maize plants that have already been infected by *Meloidogyne* spp. are also host to most fungi including *Fusarium* spp. *Phytophthora* spp., *Verticillium* spp. and *Rhizoctonia* spp. (Taylor & Sasser, 1978). The subsequent damage can disrupt and minimise the ability of the host to absorb water and minerals, and translocate it to the aerial plant parts, and finally result in greater yield losses (Meena *et al*., 2016).
Nutrition, economic and social value	More than 80% of the human population depends on maize as a food source, or a means of income to sustain their livelihoods (Sharma & Misra, 2011; Ranum *et al*., 2014; Pardey *et al*., 2016). Additionally, most of the nutrients in grain (33% fat, 19% proteins as well as minerals, vitamins B complex and vitamin E) are contained in the embryo (Ognakossan *et al*., 2018) making it a desirable source of nutrition for animals (silage or hay) and humans.

1.2.3 Sunflower *Helianthus annuus* L.

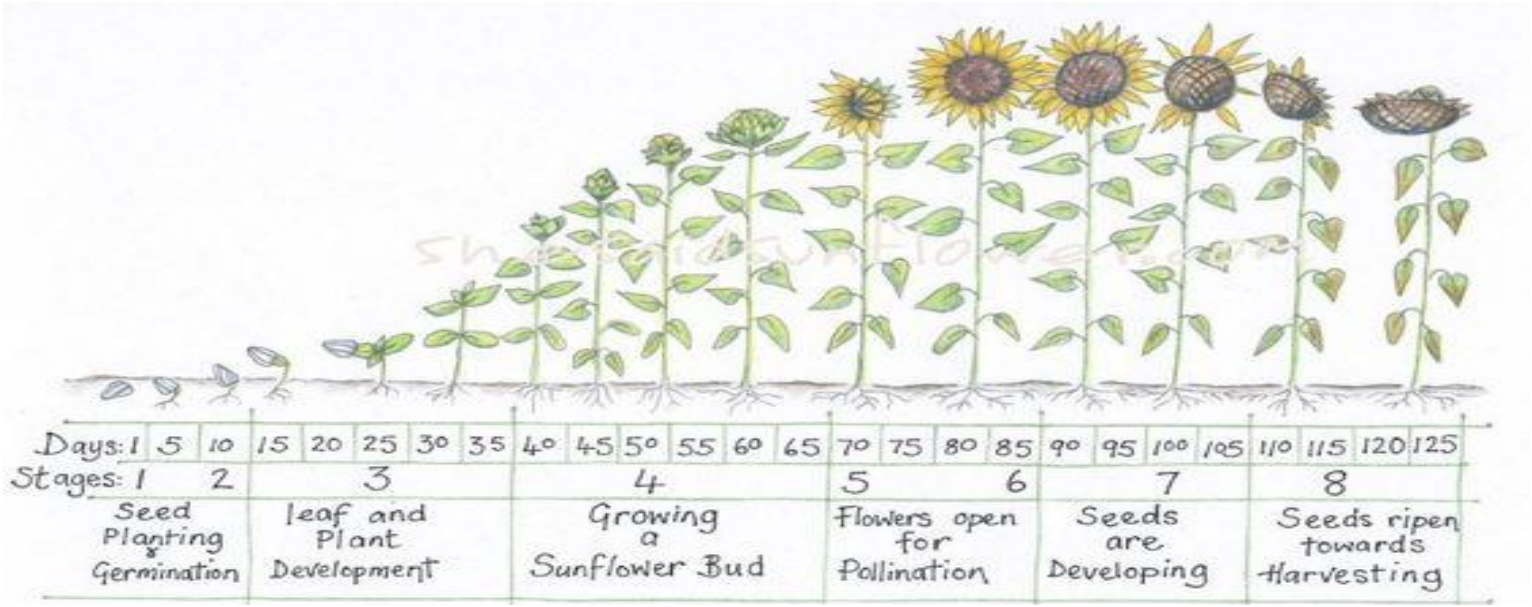
1.2.3.1. Production figures

World sunflower production exceeded 57.21 MMT during the 2021-2022 production season with the top five world countries producing it being the USA, China, Brazil, Argentina, Ukraine (Table 1.2). South Africa ranks 8th with 0.9 MMT and is the major sunflower producer on the African continent (USDA, 2022).

Table 1.4 Sunflower production figures in selected world countries for the 2021-2022 growing season (USDA, 2022).

Country	**Area on which sunflower is produced (Million hectare/ha)**	**Yield of sunflower produced (Metric tons per hectare/ha)**	**Production of sunflower (Million metric tons)**
Globally (except Africa)			
Ukraine	1.6	2.5	17.5
Russia	9.6	1.6	15.5
European Union	4.4	2.4	10.4
Argentina	1.6	2.1	3.4
United State of America	0.5	1.7	0.9
Africa			
South Africa	0.6	1.6	0.9
Morocco	0.02	1.3	0.03
Egypt	0.01	2.4	0.02

Table 1.5 A summary of basic information on sunflower and production requirements to optimize sunflower yield under South African climatic conditions.

Origin and distribution	Sunflower originated in North America. The crop was introduced into Europe through Spain in 1500 and reached Russia in the late 1800s. Selection for high oil content began in Russia in 1860 and it was raised from 28% to nearly 50% (DAFF, 2010). Russia's high-oil lines were reintroduced into the United States of America in 1893 (Putt, 1997). Sunflower cultivars with a high oil content was introduced to the rest of the world from there.
Classification	Taxonomy according to Fernández-Luqueño *et al*. (2014) Kingdom Plantae Class Magnoliopsida Superorder Asteranae Order Asterales Family Asteraceae Subfamily Helianthoideae Genus *Helianthus* Species *annuus*
Growth stages	 **Fig.1.2** A schematic illustrations of different germinantion and establishment, leaf development, flowerbud, flower and seed development stages of sunflower linked to its growth and development (Illustration: Sunflower Production – Shesaidsunflower.com).

Sunflower sectors	Sunflower is grown by both commercial and developing producers, although commercial producers produce most of the crop and dominate contribution to the market (Abafe, 2021). Sunflower is often rotated with maize, and therefore practices for sunflower are similar to those used for producing maize (see Table 1.4).
Factors influencing sunflower production	
Climatic requirements	The optimal temperature for all stages of sunflower growth is 23 - 28 °C; temperatures above 34 °C have little effect on productivity. A soil temperature range of 14 - 21 °C during planting is required for optimal seed germination. Sunflower seeds, however, can germinate at low temperatures (e.g., as low as 5 °C). Annual rainfall in the range of 400 - 600 mm is required for optimal yield and quality (GrainSA, 2018).
Fertilization	Boron (B), molybdenum (Mo), nitrogen (N), and phosphorus (P) are essential elements for optimal sunflower growth. Prior to planting, Mo should be applied to the seeds. If soil analysis is not available, 50 kg/ha to 100 kg/ha of an N: P: K/3:2:1 (25) fertiliser mixture applied at planting is sufficient for a yield potential of 1 000 - 1 500 kg/ha (DAFF, 2010).
Cultivar choice	Cultivar selection is based on one that performs optimally in the area where the producer's farm is situated and is dependent on yield and yield reliability. Other factors to consider when selecting a cultivar include oil percentage, maturity period, seed size (for non-oilseed markets), lodging and bird resistance (DAFF, 2010). In addition, grain producers plant sunflower cultivars such as AG5278, AG8251, PAN160, PAN7160CLP and others (Table 2.1a & b).
Planting time and depth	Sunflower is planted in SA from early November to the end of December in the east and mid-January in the west (DAFF, 2021). Sunflower seeds are planted at depths of 25 and 50 mm in clay soils and sandy soils, respectively. Row widths can range from 90 - 100 cm, but wider rows of 1.5 - 2.1 m can also be used, especially to accommodate farm implements (DAFF, 2010).
Other important information	
Harvesting	When the seed contains ±9 -10% moisture, harvesting should begin before the heads are completely dry. The seed moisture percentage must be closely monitored to ensure that the crop can be harvested as soon as possible (GrainSA, 2021), and this can be done manually (for developing producers) or mechanically (for larger farms) (commercial producers).
Disease and weed control	The most serious sunflower diseases are caused by fungi. The most common diseases are rust (*Pucciniales*), downy mildew (*Peronospora sparsa*), Verticillium wilt (*Verticillium dahlia*), Sclerotinia stalk (*Sclerotinia sclerotiorum*), and leaf spot (*Alternaria alternata*). Sunflower germplasm has been improved to resist rust, downy mildew, and Verticillium wilt (GrainSA, 2016). Weeds are typically controlled with herbicides, particularly broadleaf weeds, which have been a constraint for local sunflower producers (Grain SA, 2021).
PPN interactions with other pathogens	*Meloidogyne* spp., particularly *Meloidogyne arenaria* (Neal, 1889) Chitwood, 1949, *M. incognita* and *M. javanica* are significant constraints to sunflower production in SA (Van der Linde *et al*., 1959), while *Meloidogyne hapla* Chitwood, 1949has also been associated with roots of the crop (Keetch & Buckley 1984; Kleynhans *et al*., 1996). Little is known about plant-parasitic nematode (PPN) interactions with one another and with other microbe pathogens in sunflower fields (Jacksom-Ziems, 2016).
Nutrition, economic and social value	Sunflower contributes to the development of producers' household food security, while commercial producers deliver to the market (Lekunze *et al*., 2011). Sunflower oil is either sold or further processed to make margarine, cooking fat, biofuel, massage blends, and fat spread, all of which are common household items. Oilcake is another byproduct of sunflower that is used to make animal feed in the form of sunflower oilcake meal (DAFF, 2017).

1.3 Nematodes

Nematodes are unsegmented and microscopic worm-like animals that occur in various habitats in soil, water, and various other substrates (Decraemer & Hunt, 2013). Many nematodes are either parasitic to animals, humans or plants (Decraemer & Hunt, 2006).

The PPN associated with maize and sunflower crops worldwide (Amarantha & Krishnappa, 1990; Korayem *et al*., 2011; Ibrahim *et al*., 2021) and in SSA has been documented (Lawn *et al*., 1988; Fourie *et al*., 2017). Root-knot nematodes, *Meloidogyne* Göldi, 1887, are generally the economically important genus parasitizing these crops (Jones *et al*., 2013). Therefore, this review is broadly dealing with the basic information (classification, biology and morphology and damage potential) of this No-1 rated genus. In addition, *Pratylenchus*, occupying the 3rd position of the Top-10 list of economically important PPN worldwide (Jones *et al*., 2013) follows *Meloidogyne* in terms of its predominance in grain fields in SA (Mc Donald *et al*., 2017). Therefore, a concise overview for the latter genus will also be provided.

1.3.1 Classification of plant-parasitic nematodes and basic biology, and morphology

1.3.1.1 Classification

The taxonomic position of nematode taxa is determined using both classical (morphology and morphometrics) and molecular analyses (phylogeny). The systematic order classifications of *Meloidogyne* and *Pratylenchus,* are as follows according to Decraemer & Hunt (2013).

Meloidogyne:
Phylum Nematoda Potts, 1932
Class Chromadorea Inglis, 1983
Order Rhabditida Chitwood, 1933
Suborder Tylenchina Thorne, 1949
Superfamily Tylenchoidea Decraemer & Hunt, 2013
Family Hoplolaimidae Decraemer & Hunt, 2013
Subfamily Meloidogyninae Decraemer & Hunt, 2013
Genus *Meloidogyne* Göldi, 1887

Pratylenchus:

Phylum Nematoda Potts, 1932

Class Chromadorea Inglis, 1983

Order Rhabditida Chitwood, 1933

Suborder Tylenchina Chitwood, 1950

Superfamily Tylenchoidea Orley, 1880

Family Pratylenchidae Thorne, 1949

Subfamily Pratylenchinae Thorne, 1949

Genus *Pratylenchus* Filipjev, 1936

1.3.1.2 Basic biology and morphology of PPN

Plant-parasitic nematodes are motile, vermiform organisms and range in size from 0.2 - 12 mm in length and 10 - 35 mm in diameter (Kleynhans *et al*., 1996; Colombo *et al*., 2014). Typically, nematodes have an external and internal cylindrical digestive system separated by a pseudocoelomic cavity (Decreamer & Hunt, 2013), and a body wall that consists of a relatively impermeable cuticle and hypodermis facilitating a high turgor pressure system (Traunspurger, 2000).

Sexual dimorphism occurs in sessile, endoparasitic genera such as *Meloidogyne*, *Globodera* (Skarbilovich, 1959) (cyst nematodes), *Heterodera* Schmidt, 1871 (cyst nematodes), *Rotylenchulus* Linford & Oliveira, 1940 (spiral nematodes), *Tylenchulus* Cobb, 1913 (citrus nematodes) and others (Luc *et al*., 1990). In these genera the female body shapes are spherical or roundish and not worm-shaped as that of the infective, motile second-stage juvenilies (J2) and males (Dereamer & Hunt, 2013). Individuals in these latter genera are embedded entirely or partially in the roots/other below-ground parts of their hosts, where they induce complex feeding structures that provide them with a long-lasting food source (Jones *et al*., 2013; Decraemer & Hunt, 2013). Mature females of *Meloidogyne* spp. are thus sedentary endoparasites, which feeds from giant cells that started to form during the feeding of the infective J2 (Holbein *et al*., 2019).

Plant-parasitic nematodes use a stylet in the anterior part, head, of their bodies to penetrate the cell walls of host tissue and withdraw cell contents from various below-ground plant parts. Three stylet types are known to occur in PPN, namely: stomatostylet (Tylenchida,

which *Meloidogyne* spp. belongs to), onchiostylet (Triplonchida), and odontostylet (Dorylaimida), (Kumar & Yadav, 2020).

1.3.1.3 Life cycle

For root-knot nematodes, the initial juvenile stage (J1) occurs in the egg, where it goes through its first moult before emerging as the motile J2 (Decraemer & Hunt, 2006; Hunt *et al*., 2018; Kleynhans, 1999). The infectious stage of this genus is the J2, which infects the roots of a host plant. It then develops into a swollen J2, a swollen third-stage juvenile (J3), and finally a swollen fourth-stage juvenile(J4). The latter two stages are not feeding and lack stylets. After a final moult, the J4 either matures into an adult motile male or bloated female. Stylets are present in both males and females, but only the female actively feeds (Decraemer & Hunt, 2006; Hunt *et al*., 2018). Fig.1.3 depicts the life cycle of RKN.

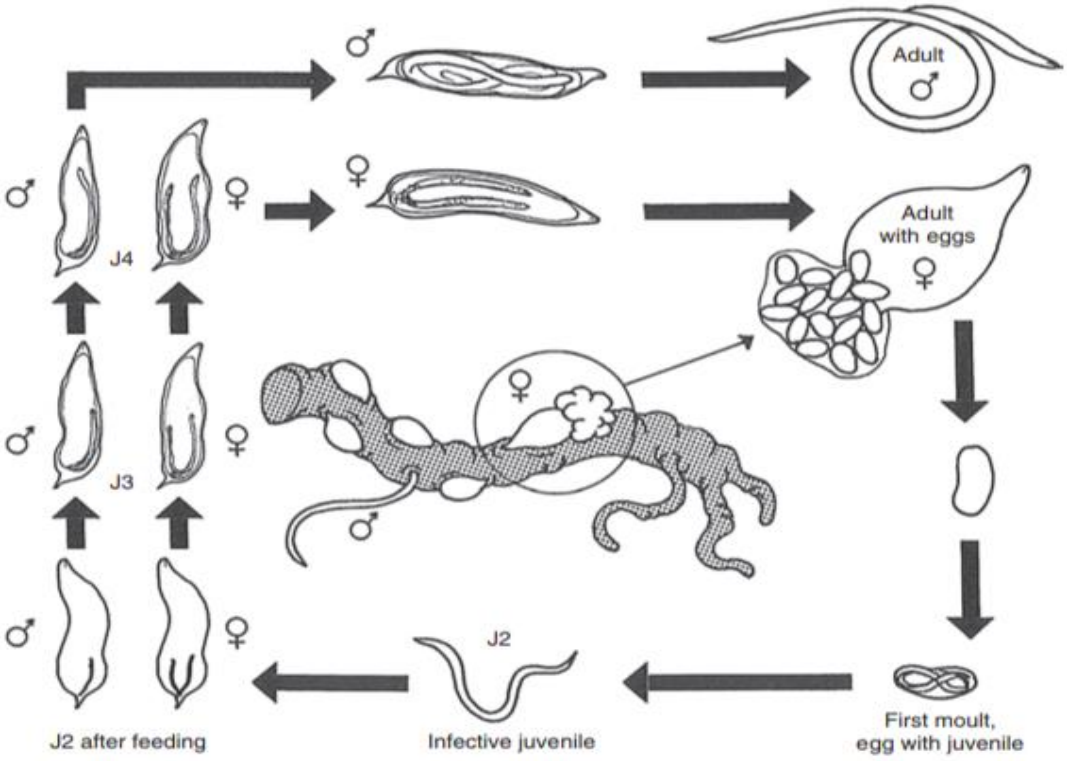


Fig.1.3 The life cycle of root knot nematode with the roots of a host plant (Karssen and Moens, 2006; Moens, 2009).

1.3.1.4 Economically most important plant-parasitic nematodes associated with maize and sunflower in South Africa

Although *Meloidogyne* is reported as the economically most important PPN that infect and parasitise grain crops in SA, it is most likely to be present simultaneously with *Pratylenchus* spp. in grain fields in SSA (Mc Donald *et al*., 2017; Coyne *et al*., 2018). In Uganda, 28% of yield losses were reported in maize fields infested with a mixed community of *Meloidogyne* and *Pratylenchus* spp. (Kagoda *et al.,* 2011). Locally, *Meloidogyne* spp*.* followed by *Pratylenchus* spp*.,* which are also considered the economically most important nematode pests in maize-cropping systems, cause substantial yield losses to maize and sunflower crops (Mc Donald *et al.,* 2017; Fourie *et al.,* 2017; Mbatyoti *et al.,* 2020).

Meloidogyne javanica (Treub, 1885) Chitwood, 1949, and *Meloidogyne incognita* (Kofoid and White, 1919) Chitwood, 1949, are the most common and predominant *Meloidogyne* spp. infecting maize and sunflower in SA (Mc Donald *et al.,* 2017), with *Meloidogyne arenaria* (Neal, 1889) Chitwood, 1949, and *Meloidogyne hapla* Chitwood, 1949, also present in local fields, but to a lesser extent (Mc Donald *et al.,* 2017; Fourie, 2022). In terms of *Pratylenchus*, the predominant species infecting maize and sunflower in SA are: *Pratylenchus brachyurus* (Godfrey, 1929) Filipjev & Schuurmans-Stekhoven, 1941; *Pratylenchus penetrans* (Cobb, 1917) Filipjev & Schuurmans-Stekhoven, 1941; *Pratylenchus pratensis* (de Man, 1880) Filipjev, 1936; and *Pratylenchus zeae* Graham, 1951 (Mc Donald *et al*., 2017; Fourie *et al*., 2017). Species belonging to these two PPN genera infect a wide range of crops used in crop rotation sequences in local grain fields (Mc Donald *et al*., 2017; Van den Berg *et al*., 2017). As a result, in SA there are no available non-host crops that can be used in grain fields to combat *Meloidogyne* spp. (Fourie, 2022).

In the following part, *Meloidogyne* and *Pratylenchus* spp. are summarized in tabular form with reference to the following aspects: predominant species associated with maize, body form, distribution, host range, symptoms and damage, life cycle, reproduction strategies and interaction with other soil organisms.

Table 1.6 A summary of predominant species of *Meloidogyne* and *Pratylenchus* identified in South African maize and sunflower fields, with concise notes on their distribution, host range, symptoms and damage inflicted in host plants, biology and interaction with other soil organisms.

	Meloidogyne **(root-knot nematodes/RKN)**	***Pratylenchus*** **(root-lesion nematodes/RLN)**
Predominant species	Predominant species in maize fields are *Meloidogyne incognita*, *M. javanica* followed by *M. arenaria* and *M. enterolobii* (Mc Donald *et al*., 2017; Fourie *et al*., 2017). Predominant species in sunflower fields are *Meloidogyne arenaria*, *M. incognita* and *M. javanica* (Van der Linde *et al*., 1959).	Predominant species in maize fields are *Pratylenchus brachyurus*, *P. penetrans*, *P. pratensis* and *P. zeae* (Mc Donald *et al*., 2017; Fourie *et al*., 2017), *P. delattrei* , *P. neglectus* (De Waele & Jordaan,1988; SAPPNS database as referred to by Marais, 2017), Predominant species in sunflower fields are *Pratylenchus brachyurus*, (Koen, 1967), *P. penetrans*, *P. pratensis* and *P. zeae* (Mc Donald *et al*., 2017; Fourie *et al*., 2017).
Distribution	All provinces of South Africa (Kleynhans *et al*., 1996; Onkendi & Moleleki, 2013; Agenbag, 2016).	All provinces of South Africa (Kleynhans *et al*., 1996; Fourie *et al*., 2001).
Host range	Wide host range infecting agricultural and horticultural crops and weeds (Whitehead, 1969; IITA, 1981; CABI, 2002b; CABI, 2003;CABI, 2005; SAPPNS database as referred to by Marais, 2017).	Wide host range infecting agricultural and horticultural crops and weeds (Kleynhans *et al*., 1996; Fourie *et al*., 2001; CABI, 2002b; CABI, 2005; SAPPNS database as referred to by Marais, 2017).
Symptoms and damage	 **Fig.1.4** Characteristic gall formation on maize roots caused by root-knot nematode feeding (Courtesy: Raymond Collet, NWU).	 **Fig.1.5** Dark lesions on maize roots caused by lesion nematode feeding (Courtesy: Danny Coyne, IITA).
Body form	Sexual dimorphism: vermiform, infective second-stage juveniles and males, but globose third and fourth stage juveniles as well as females (Karssen *et al*., 2013).	Verfmiform: juveniles and adults (Fosu-Nyarko & Jones, 2016).
Feeding behaviour	Sedentary endoparasites in plant roots/other below plant parts (Holbein *et al*., 2019).	Migratory endoparasites in plant roots/other below plant parts (Fosu-Nyarko & Jones, 2016)
Life cycle	Between 20 - 30 days at optimum temperature of 25 - 30 °C (Greco & Di Vito, 2009; Shurtleff & Averre, 2000).	Between 3 - 8 weeks (Jones & Fosu-Nyarko, 2014).
Reproduction	Reproduction is either amphimixis, facultative	Reproduction is mainly through

strategies	meiotic parthenogenesis or obligatory meitotic parthenogenesis (Chitwood & Perry 2009). *M. arenaria*, *M. enterolobii*, *M. incognita* and *M. javanica* reproduce by obligatory mitotic parthenogenesis (Chitwood & Perry 2009). *M. hapla,* reproduces either facultative meiotic parthenogenesis or obligatory mitotic parthenogenesis (Chitwood & Perry 2009).	parthenogenesis and amphimixis (Jones & Fosu-Nyarko, 2014).
Interaction with other soil organisms	Damaged root tissues of maize plant attributed to *Meloidogyne* spp. infection are vulnerable to bacteria infestation including *Pseudomonas* spp., *Agrobacterium* spp. and *Corynebacterium* spp. (Taylor & Sasser, 1978). Infected maize roots also host most fungi including *Fusarium* spp., *Alternaria* spp., *Phytophthora* spp., *Verticillium* spp. and *Rhizoctonia* spp. (Taylor and Sasser, 1978).	*Pratylenchus* spp. commonly interact with the wilt fungii *Fusarium* and *Verticillium* and with other root-rot pathogens such as *Pythium Pringsheim*, *Rhizoctonia* and *Phytophthora* (Back *et al*., 2002).

1.3.1.4 Other plant parasitic nematodes associated with maize and sunflower

Maize can be infected by more than 60 PPN species (Dababat & Fourie, 2018). The economically most important genera, *viz. Meloidogyne* and *Pratylenchus* infecting maize and sunflower, other genera that are reported to infect these crops amongst others are *Aphelenchoides* Fischer, 1894, *Ditylenchus* Filipjev, 1936, *Helicotylenchus* Steiner, 1945, *Lobocriconema* de Grisse & Loof, 1965, *Longidorus* Micoletzky, 1922, *Nanidorus minor* Colbran, 1956, *Paratrichodorus* Siddiqi, 1974, *Rotylenchulus*, *Rotylenchus* Filipjev, 1936, *Scutellonema* Andrássy, 1958, *Telotylenchus* Siddiqi, 1960 and *Tylenchorhynchus* Cobb, 1913 (Louw, 1982; De Waele & Jordaan, 1988a; Nicol *et al*., 2011; Mary *et al*., 2013). In addition, no damage inflicted by the latter PPN has been recorded by local maize producers and nematologists, their population densities are generally low and they are only found sporadically in some grain fields (Bekker, 2016; Pretorius, 2018). Nevertheless, researchers should keep monitoring population densities of all PPN genera and species found in maize and sunflower rhizospheres on a basis. For maize, the genus *Heterodera* Inchohe, 1952 that infect and cause considerable damage to maize in eastern world countries, have not been listed for SA (Dababat & Fourie, 2018). Furthermore, interestingly *Meloidogyne* and *Hoplolaimus* von Daday, 1905 are the most frequently reported genera on maize in the USA (Koenning *et al*., 1999), while *Meloidogyne* and *Pratylenchus* are commonly recorded for SA (Mc Donald *et al*., 2017).

Since it is hypothesised that *Meloidogyne* will be the predominant PPN infecting maize and

sunflower in localities sampled during this survey, the next part of the literature review will focus primarily on the former genus.

1.4 Identification of *Meloidogyne* spp.

Meloidogyne is recorded as the No-1 nematode pest worldwide causing great economic damage to various crops (Jones *et al*., 2013). As a result, correct identification of *Meloidogyne* spp. is of paramount significance in selecting an integrated approach to enable a reasonable degree of nematode pest management (Hunt & Handoo, 2009). For example, worldwide, 105 *Meloidogyne* spp. were identified by 2020 (Ghaderi & Karssen, 2020). From the African continent, 23 *Meloidogyne* spp. have been identified (dos Santos *et al*., 2018), while 14 have been reported to occur in SA (Marais *et al*., 2017). Existing methods for identifying *Meloidogyne* spp. include morphology and morphometrics (Karssen, 2002), isozyme phenotyping (Esbenshade & Triantaphyllou, 1987; da Cunha *et al*., 2018; dos Santos *et al.*, 2019), and from the late 1990s molecular diagnostics gained popularity (Hunt & Handoo 2009; Moens *et al.,* 2009; da Cunha *et al.,* 2018).

1.4.1 Morphological and morphometrical identification

Studying perineal-pattern characteristics of mature females, e.g., apex, dorsal arch, lateral lines, phasmids, striae, and tail regions (Table 4.1) were the primary focus of the classical approach (morphological and morphometrical identifications) in attempting to elucidate the identity of *Meloidogyne* spp. (Yang & Eisenback, 1983; Kleynhans, 1991; Brito *et al*., 2004). Kleynhans (1991) studied the shapes of the oesophageal linings, metacorpus and procorpus of mature, South African *Meloidogyne* spp. females to complement perineal-pattern analysis and aid in identifying species. However, using only the classical approach in discrimination among species has been a challenge, with no information being published, for example, on the oesophageal characteristics of some species, particularly *M. enterolobii* (Kleynhans, 1991). Furthermore, it is challenging to identify *M. enterolobii* using morphological features only since several of its characteristics are overlapping with its counterpart, thermophilic species (Carneiro *et al*., 2016), particularly *M. incognita*. This challenge applies even for experienced and expert taxonomists.

Although a few records have been published by molecular biologists and nematologists, from the 1980s onwards until 2000, applying biochemical and molecular techniques to

complement classical attempts in accurately identifying *Meloidogyne* spp. (Yang and Eisenback, 1983; Curran *et al.,* 1985; Curran *et al*., 1986; Blok *et al*., 1997; Zijlstra *et al*., 1997; Zijlstra, 2000), their application became increasing popular from the early 2000s (Dong *et al*., 2001; Blok *et al*., 2002; Powers *et al*., 2005; Karssen *et al*., 2013; Guzman *et al*., 2021). In Africa this approach is also gaining popularity with more and more records becoming available about the identity of *Meloidogyne* spp. This especially applies to the four economically important ones namely *Meloidogyne enterolobii* Yang & Eisenback*,* 1983, *M. arenaria, M. incognita* and *M javanica* (Fourie *et al*., 2001; Berry *et al*., 2008; Ntidi *et al*., 2012; Onkendi & Moleleki 2013a, b; Visagie *et al*., 2018; Dos Santos *et al*., 2019; Rashidifard *et al*., 2018b).

For this study, mature females of *Meloidogyne* spp. were obtained from *in vivo* reared populations (containing one species) or communities (containing more than one species) (Rashidifard *et al*., 2019a & b; Bello *et al*., 2020) that were originally sampled from maize and sunflower fields of 15 New-Era grain producers (see section 2.3) and identified by complementing classical morphometrics (perineal-pattern morphology and oesophageal characteristics) with the use of the molecular technique, SCAR-PCR.

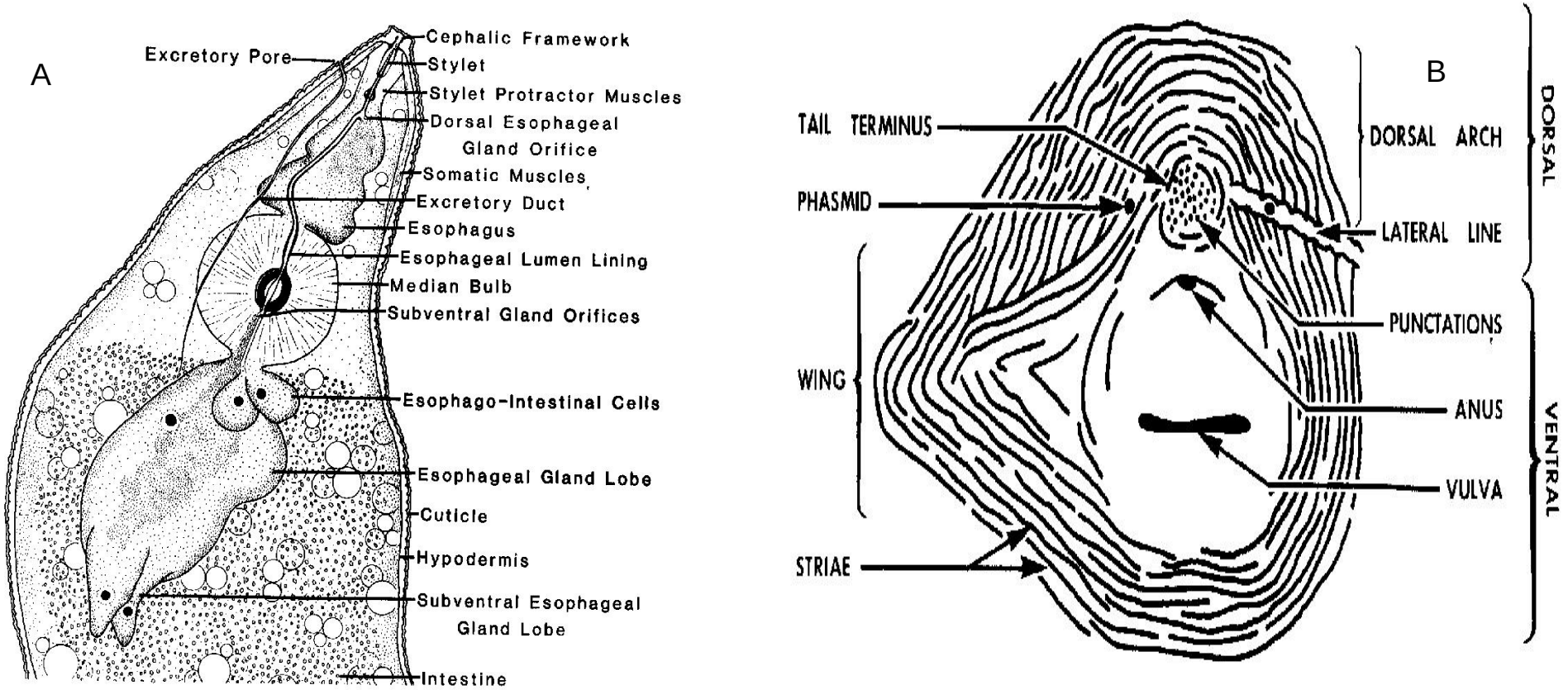


Fig.1.6 Female morphology of *Meloidogyne* spp. **(A)** anterior Region and **(B)** perineal pattern in the posterior region of a female (Obtained from Eisenback & Triantaphyllou, 1991).

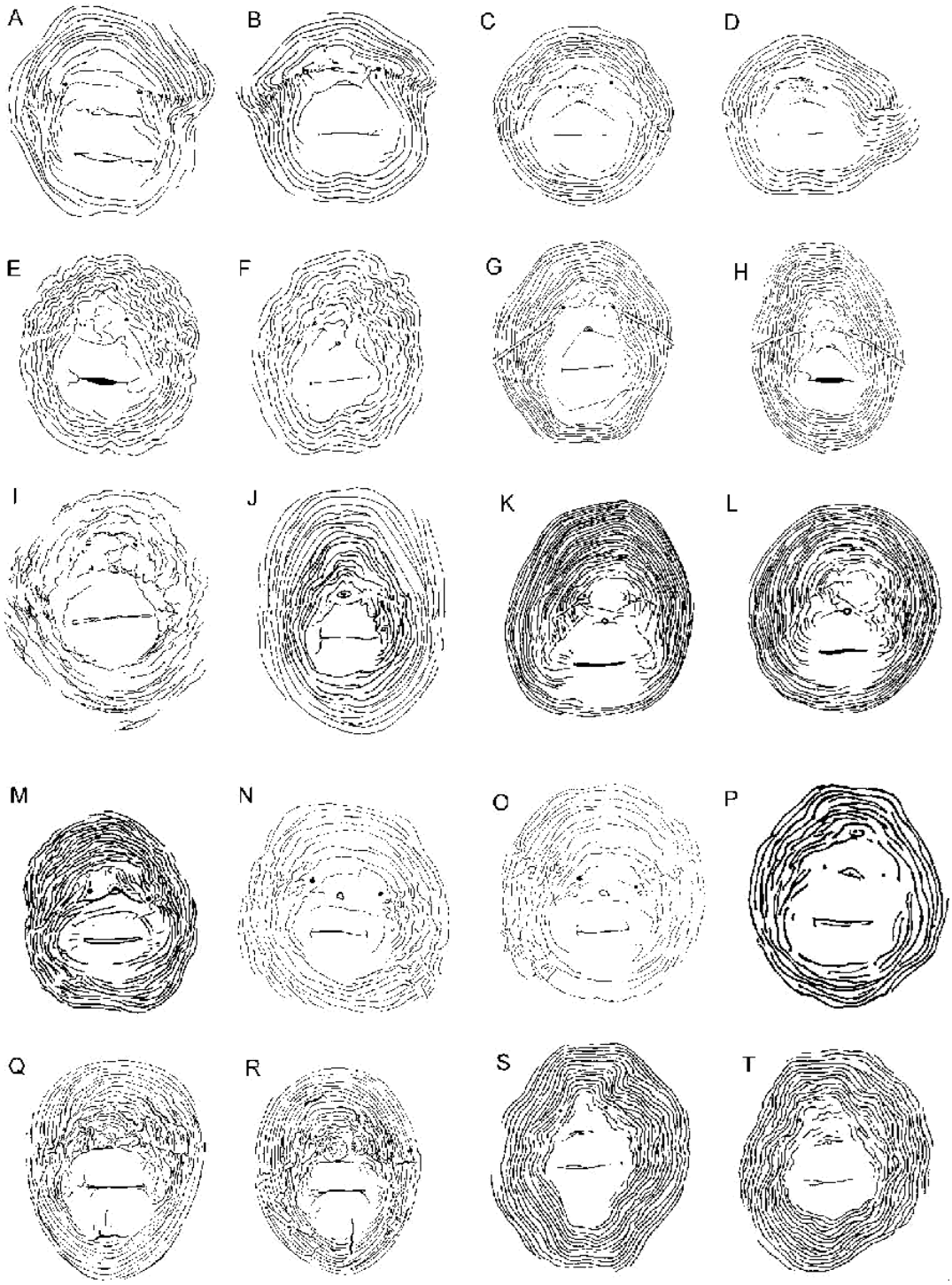


Fig. 1.7 Variation of perineal patterns among some *Meloidogyne* spp. females. **A, B**: *M. arenaria*; **C**, **D**: *M. hapla*; **E**, **F**: *M. incognita*; **G**, **H**: *M. javanica*; **I**: *M. acronea*; **J**: *M. chitwoodi*; **K**, **L**: *M. enterolobii*; **M**: *M. ethiopica*; **N**, **O**: *M. exigua*; P: *M. fallax*; **Q**, **R**: *M. graminicola*; **S**, **T**: *M. paranaensis* (Obtained from Hunt & Handoo, 2009).

For a long time, most laboratories have relied on the morphological identification of female perineal patterns to identify *Meloidogyne* spp. (Carneiro *et al*., 2017). In South Africa, various studies reported on the morphological identification of species with reports by Kleynhans (1991), representing a valuable baseline set containing extensive morphological and morphometrical descriptions of a wide range of species. Other useful contributions in this specific field are contained in another publication by Kleynhans *et al*. (1997), titled "Collecting and preserving nematodes". Also, Rashidifard *et al*. (2019), contributed extensively to identification of 48 populations (representing single species) and/or communities (containing a mixture of species) of *Meloidogyne* to species level using this classical approach. However, morphology and morphometrics cannot be used as the sole criteria as it does not sufficiently discriminate in distinguishing key features that are essential for the precise distinction of cryptic species (Jones & Fosu-Nyarko, 2014), such as *M. enterolobii* (Yang & Eisenback,1983) and its thermophilic counterparts (*M. arenaria*, *M. incognita*, *M. javanica* & others). Morphology and morphometric identification alone have proven to be difficult, and in certain cases impossible, for the latter species (Visagie *et al*., 2018; Rashidifard *et al*., 2019). For example, *M. enterolobii* is likely to be confused with especially *M. incognita* due to characteristic overlapping morphological similarities such as similar female perineal patterns and slight differences on the heads of females (Brito *et al.,* 2004; Block & Power, 2009; Hunt & Handoo, 2009; Pretorius, 2018). The same applies to the cryophilic species *M. chitwoodi*, *M. fallax* Karssen, 1996 and *M. hapla* that also are difficult to distinguish from each other due to similar morphological and morphometrical characteristics they share.

An interesting and valuable contribution to identify *Meloidogyne* spp. using classical techniques was recently published by Ghaderi & Karssen (2020) using various morphology and morphometric parameters, e.g., based on the length and shape of the tail tip of J2 and certain male characteristics. Rashidifard *et al*. (2019c) on the other hand demonstrated three characteristics of the perineal patterns of mature females that can be used to distinguish the South African *M. enterolobii* populations from its thermophilic counterpart species: large phasmids surrounded by fine striae; fine striae on the lateral sides of the vulva; and the perineal pattern of mature females with a medium to high square-like dorsal arch.

1.4.2 Molecular and biochemical identification of *Meloidogyne* spp.

Several studies including a recent one done by Bogale *et al*. (2020) proved that it is challenging to use morphological and morphometrical approaches alone in accurately identifying *Meloidogyne* spp., that closely resemble each other in terms of their characteristics. As a result, researchers have developed more specialised molecular techniques that have become reliable, namely isozyme or esterase phenotyping (Esbenshade & Triantaphyllou, 1985), molecular diagnostics (Hunt & Handoo, 2009; Moens *et al*., 2009; Rashidifard *et al*., 2019a) and various genetic approaches (Rashidifard *et al*., 2018; Koutsovoulos *et al*., 2020) which can, and should be integrated with morphological and morphometrical approaches.

Sequence characterised amplified regions - polymerase chain reaction (SCAR-PCR) techniques were used to identify *Meloidogyne* spp. found infecting maize and sunflower roots at the localities sampled as part of this study (see Chapter 3), this approach will be concisely explained and focused on below.

1.4.2.1.1 Sequence characterised amplified regions – polymerase chain reaction (SCAR-PCR)

The sequenced characterised amplified region-specific primers are derived from sequences of species-specific randomly amplified polymorphic DNA (RAPD markers, such as thode of *M. arenaria*, *M. chitwoodi*, *M. enterolobii*, *M. fallax*, *M. hapla*, *M. incognita*, and *M. javanica* (Blok & Powers, 2009). The specificity and sensitivity of this method can identify multiple *Meloidogyne* spp. in a single reaction; however, interference between primers can occur (Blok & Powers, 2009). In South China, multiplex PCR and DNA extract successfully identified species specific primers for *M. incognita*, *M. enterolobii*, and *M. javanica* (Hu *et al.,* 2011).

This technique is generally feasible and cost effective primarily for identification of *Meloidogyne* spp. (Onkendi *et al*., 2014), and faster for identification of nematodes at any stages of nematode life cycle (Zijlstra, 2000). Therefore, it is preferred by most researchers (Rashidifard *et al*., 2019). Moreover, Rashidifard *et al*. (2019) proved that SCAR-PCR can be a highly efficient tool for identifying *Meloidogyne* spp. from single-species populations and/or mixed communities. Furthermore, in SA, the SCAR-PCR technique was successfully

used by various scientists to identify *M. arenaria*, *M. chitwoodi*, *M. enterolobii*, *M. fallax*, *M. hapla*, *M. incognita*, and *M. javanica* in agricultural fields (Fourie *et al*., 2001; Onkendi & Moleleki, 2013; Ntidi, 2016; Rashidifard *et al*., 2018; Visagie *et al*., 2018).

Figure 1.8 indicates the DNA bands amplified using the SCAR-PCR technique for identification of *Meloidogyne* spp. with the 1-Kb size marker (M) and DNA of *M. javanica*; *M. arenaria*; and *M. incognita* shown and representing well F1, F2 and F3 containing bands of these species.

One disadvantage of SCAR-PCR is its inability to provide precise identification of new and/or emerging *Meloidogyne* spp., for example, the use of SCAR-PCR markers alone produced inaccurate results since it failed identifying *M. ethiopica* and two atypic, unknown *Meloidogyne* spp.

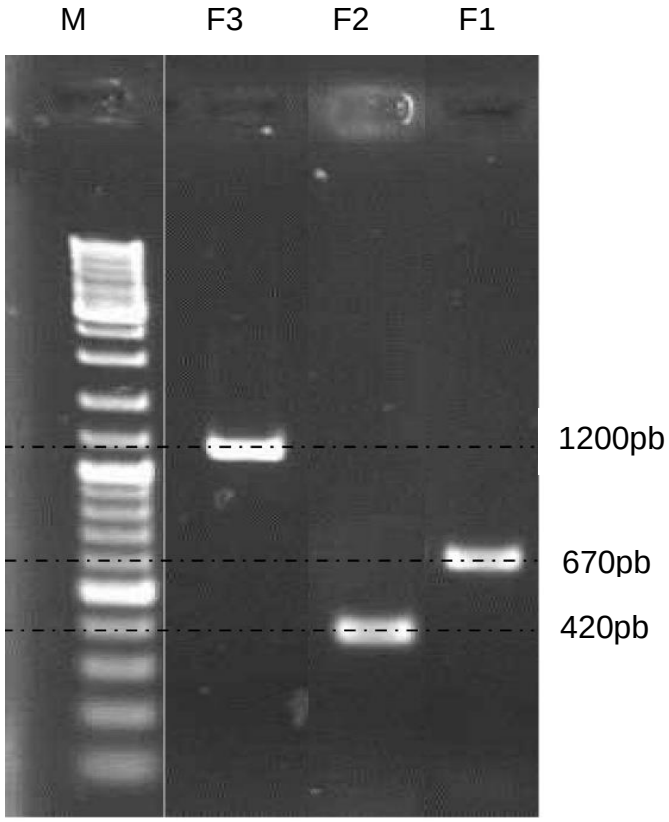


Fig. 1.8 Gel photograph of deoxyribonucleic acid amplification products of *Meloidogyne* spp. females using the sequence characterised amplified region – polymerase chain reaction (SCAR-PCR). M = 1-Kb size marker DNA, F1 = *M. javanica*, F2 = *M. arenaria*, F3 = *M. incognita*) (Obtained from Tavoillot *et al*., 2021).

Other molecular techniques in addition to SCAR-PCR have been used over the past 25 years to identify *Meloidogyne* spp. (Cenis *et al*., 1992; Powers & Harris 1993; Stanton *et al.*, 1997; Zijlstra *et al*., 1997; Zijlstra *et al*., 2000; Blok 2005; Adam *et al*., 2007; Landa *et al*., 2008; Blok & Powers 2009; Tigano *et al*., 2010; Akyazi & Felek 2013; Janssen *et al*., 2016). Researchers have focused on ribosomal DNA (rDNA) (e.g., ITS and the D2-D3 segment of 28S), mitochondrial DNA (mtDNA) (specifically, Cytochrome oxidase 1: COI, COII/16S, NADH5 mitochondrial DNA (mtDNA), Intergenic Spacer (IGS-2) regions, Random Amplified Polymorphic DNA (RAPD) Rutter *et al*. (2019), Santos *et al*. (2019), and others. For example, a study by Onkendi & Moleleki (2013b) revealed that *M. enterolobii* belonged to one clade, while *M. arenaria*, *M. incognita*, and *M. javanica* belonged to another using IGS-rDNA and mtDNA sequences. However, these authors discovered that *M. enterolobii* and *M. incognita* belonged to the same clade, whereas *M. arenaria* and *M. javanica* belonged to different clades based on their analysis of the D2-D3 gene (28S rDNA).

1.4.2.1.3 Other molecular methods used to identify *Meloidogyne* spp.

In the table below other sophisticated, state-of-the-art molecular-based techniques (not including the SCAR-PCR and classical techniques referred to above) that are used to identify *Meloidogyne* spp. across the globe are listed.

Table 1.7 Molecular and/or genetic techniques used to identify *Meloidogyne* spp.

Technique	Reference
Protein-based analyses: isozymes	
Enzyme phenotyping using isozyme patterns of esterases (EST	Yang (1984), Esbenshade & Triantaphyllou (1985), Fargette (1987), Fargette & Braaksma (1990), Brito *et al*. (2004), Carneiro *et al.* (2000), Randig *et al*. (2004), Hernandez *et al.* (2004), Torres *et al*. (2004), Xu *et al*. (2004), Lima *et al*. (2005), Liu *et al*. (2005), Lugo *et al.* (2005), Molinari *et al.* (2005), Kaur *et al*. (2006); Brito, Stanley, Kaurm *et al*. (2007), Brito, Paes *et al*. (2012), Rosa et al. (2013) Rosa, Oliveira *et al.* (2014) Villain *et al.* (2013), Machado & Filho (2014), Paes-Takahashi *et al*. (2015), Wang *et al.* (2015), Janssen *et al.* (2016), da Silva *et al*. (2016), Kolombia *et al*. (2016, 2017), Freitas *et al.* (2017), Bellé *et al*. (2018), da Cunha *et al.* (2017), dos Santos *et al*. (2019), Lu *et al*. (2019), Santos *et al*. (2019)

Glutamate-oxaloacetate transaminase (GOT)	Esbenshade &Triantaphyllou (1985), Carneiro *et al*. (2000), Hernandez *et al*. (2004), Oliveira *et al*. (2007), da Cunha *et al*. (2017)
Malate dehydrogenase (MDH)	Esbenshade & Triantaphyllou (1985), Brito *et al*. (2004), Hernandez *et al*. (2004), Xu *et al*. (2004), *et al*. (2005), Molinari *et al*. (2005), Kaur *et al*. (2006); Kaurm *et al*. (2007), Oliveira *et al.* (2007), Kiewnick *et al*. (2008), Silva *et al*. (2010), Zhuo *et al.* (2010), Quénéhervé *et al*. (2011), da Silva *et al.* (2014), Wang *et al*. (2015), Kolombia *et al*. (2016; 2017), da Cunha *et al*. (2017)
Superoxide dismutase (SOD)	Esbenshade & Triantaphyllou (1985), Carneiro *et al*. (2000), Hernandez *et al.* (2004), Lugo *et al.* (2005), Molinari *et al*. (2005), Oliveira *et al*. (2007), da Cunha *et al.* (2017)
DNA-based analyses	
Mitochondrial DNA (mtDNA)	
NADH dehydrogenase subunit 5 (NADH5)	Janssen *et al*. (2016), Kolombia *et al*. (2017), Pretorius (2018), Chitambo *et al*. (2019), Rashidifard *et al.* (2018); Rashidifard *et al*. (2019a)
Cytochrome c oxidase subunit 1 (COI/CO1/COX 1)	Xu *et al*. (2004), Kiewnick *et al*. (2008), Kiewnick *et al*. (2015), da Cunha *et al.* (2017), Powers *et al*. (2018), Chitambo *et al*. (2019), Rashidifard *et al*. (2019a); Santos *et al*. (2019), Moore *et al*. (2020)
Cytochrome c oxidase subunit 2 (COII/CO2/COX 2)	Brito *et al.* (2004), Xu *et al*. (2004), Powers *et al.* (2005), Zhuo *et al.* (2010), Onkendi & Moleleki (2013a & 2013b), Ramírez-Suárez *et al.* (2014), Wang *et al*. (2014), Kolombia *et al*. (2016), Assoumana *et al*. (2017), da Cunha *et al.* (2017), Xiao *et al*. (2018), Rutter *et al.* (2019), Santos *et al.* (2019), Moore *et al*. (2020)
Cytochrome c oxidase subunit 3 (COIII/CO3/COX 3)	Janssen *et al*. (2016)
COII/16S rRNA or COII/LRNA	Blok *et al*. (2002), Brito *et al.* (2004), Powers *et al.* (2005), Zhuo *et al*. (2010), Onkendi & Moleleki (2013a &2013b), Ramírez-Suárez *et al*. (2014), Assoumana *et al*. (2017), Rashidifard (2019); *et al*. (2019a); Santos *et al*. (2019)
Ribosomal DNA (rDNA)	
External transcribed spacer (ETS)	Xu *et al*. (2004)
Intergenic spacer (IGS)	Blok *et al*. (1997), Tigano *et al*. (2010), Onkendi & Moleleki (2013a, 2013b), Ye *et al*. (2013), Long *et al.* (2015), Assoumana *et al*. (2017)

Internal transcribed spacer (ITS)	Brito *et al*. (2004), Kiewnick *et al.* (2008), Ye *et al* (2013), Wang *et al*. (2014; 2015), Filho *et al.* (2016), Villar-Luna *et al*. (2017), Lu *et al*. (2019)
Large subunit (D2-D3 28S)	Ye *et al*. (2013), Onkendi & Moleleki (2013a), Ramírez-Suárez *et al.* (2014), Xiao *et al.* (2018), Rashidifard *et al.* (2019)
Small subunit (18S)	Blok *et al*. (1997), Brito *et al*. (2004), Powers *et al*. (2005), Lu *et al*. (2019)
Other methods	
Amplified fragment length polymorphism (AFLP)	Tigano *et al*. (2010)
DNA microarrays	da Cunha *et al.* (2017)
Genotyping by Sequencing (GBS)	Rashidifard *et al.* (2018)
Inter-simple sequence repeat (ISSR)	Tigano *et al*. (2010)
iRNA	Blok *et al*. (2002), Wang *et al*. (2014)
Multiplex-PCR	Hu *et al*. (2011)
Lateral flow dipstick (LFD)	Niu *et al*. (2012)
Loop-mediated isothermal amplification (LAMP)	Niu *et al*. (2012), da Cunha *et al*. (2017), Zhou *et al*. (2017)
Random amplified polymorphic DNA (RAPD)	Carneiro *et al*. (2004a), Adam *et al*. (2007), Tigano *et al*. (2010), da Silva *et al*. (2014), dos Santos *et al*. (2019)
Real-time PCR (qPCR)	da Cunha *et al*. (2017), Kiewnick & Braun-Kiewnick (2015), Braun-Kiewnick & Kiewnick (2018)
Recombinase polymerase amplification (RPA)	Ju *et al.* (2019)
Random amplified polymorphic DNA (RAPD)	Carneiro *et al.* (2004a), Adam *et al*. (2007), Tigano *et al*. (2010), da Silva *et al.* (2014), dos Santos *et al*. (2019)
Satellite DNA (satDNA)	Randig *et al*. (2009)

1.5 Abiotic parameters influencing root-knot nematodes

Abiotic and biotic factors influence nematode diversity and population density, with PPN impacting differently on crop yield under different conditions (Abd-Elgawad & Askary, 2020). Abiotic factors including soil pH, soil moisture content, soil texture, organic matter (Cadet & Spaul, 2003), as well as climatic factors such as extreme temperatures, relative humidity influence the diversity, geographical distribution, abundance, prevalence and survival of soil nematodes (McSorley, 2003; Warner, 2009; Askary *et al*., 2012). The ability of PPN to penetrate and develop within the tissues of a host plant is, for example, strongly influenced by temperature amongst other factors (Jones *et al*., 2017b). *Meloidogyne* spp. can complete their life cycle under temperatures ranging from 25 - 30 °C in 20-30 days (Shurtleff & Averre, 2000; Greco & Di Vito, 2009).

Furthermore, *Meloidogyne* spp. generally prefer warm, moist, sandy soil conditions (Yigezu Wendimu, 2021). For example, Zhao-hui *et al*. (2006) reported that the optimal hatching temperature for *M. incognita* J2 is 15 - 30 °C, and occurs in wet sandy soil (Karssen *et al*., 2013). The J2 requires soil moisture levels ranging from 10 - 30% to optimally execute their activities, e.g., movement and infection of host plants (Yigezu Wendimu, 2021).

Various studies including Alby *et al*. (1983); Edwards *et al*. (1988); Govaerts *et al*. (2006) and Parmalee & Alston (1986) have determined the influence of various soil parameters on the abundance and diversity of nematodes. For example, abundance of *Meloidogyne* spp. have been found to be higher in sandy soils than in clay and silt soils (Jaraba-Navas *et al.,* 2007) and high population densities of *P. penetrans* was found in soils with high sand contents too (Florini *et al*., 1987). In addition, certain soil nematodes can tolerate a wide pH range, while the densities of others are adversely affected by pH, for example, alkalisation to a pH of 11 killed RKN J2 (Oka, 2010).
Since associations between nematodes and various biotic and abiotic factors often yields interesting and valuable information, the abiotic data obtained during this study were investigated using multivariate analysis to obtain a better understanding of their effects on nematode assemblages in grain fields as is discussed in chapter 3, section 3.4.

1.5 Management of root-knot nematodes

Complete eradication of PPN from a field is impossible (Kumar & Yadav, 2020). Effective management strategies, however, are fundamental (Briar *et al*., 2016), for optimal yield production and to enable food security. The goal is to reduce population densities below the economic threshold (Moens *et al.,* 2009) once the target species has been accurately identified. Early diagnosis of existing infestations, such as that of *Meloidogyne* spp., can lead to timely implementation of management strategies and lead to reduced crop losses (Kayani *et al*., 2018).

In South Africa, *Meloidogyne* spp. infecting maize roots are generally controlled using synthetic nematicides (Mc Donald & Nicol, 2005), but nematicides kill other non-target species including beneficial nematodes and other soil microorganisms (Khan *et al*., 2019). For sunflower, PPN are generally controlled using the products containing the a.s carbofuran, which is registered as an insecticide on sunflower (Van Zyl, 2013) by local pesticide producers. The use of nematicides can cause temporal depletion of *Meloidogyne* spp. in infested fields on the short term only and is an expensive strategy (Sorribas *et al*., 2005). Other strategies to control *Meloidogyne* spp. are cultural control, biological control, and genetic host plant resistance (Fourie & De Waele, 2019). This section below attempted to describe further the types of management strategies used to reduce densities of *Meloidogyne* spp. and other PPN in maize and sunflower fields.

1.5.1 Chemical control

Nematicide application is generally still the most common technique used by commercial grain producers, especially on irrigated fields (Mc Donald *et al*., 2017). Most of the synthetically derived nematicides that are approved for use in local maize fields (Agri-Intel, 2022), as well as carbofuran-containing products used as an insecticide on sunflower (Agri-Intel, 2022), which benefits producers. The use of these nematicides has significantly reduced the PPN damage, caused predominantly by *Meloidogyne* spp. associated with maize plants growing in sandy soils (Mc Donald *et al*., 2017). However, no data on the efficacy of nematicides on sunflower in SA exists (Personal communication, Driekie Fourie, NWU, Potchefstroom, September 2022).

A field study by Walter (1979b) proved that the application of carbofuran in fields infested with *P. zeae* in the Free State province increased maize production yield by 28 - 48%. In this same study in the Free State province, a 14 - 60% increase in maize production was reported on fields fumigated with DD®. Another study showed that the use of products with active substances including cloetocarb and carbofuran significantly reduced nematode pest complexes (consisting of Dorylaimidae De Man, 1876, *Meloidogyne* spp., *Paratrichodorus* spp. and *Pratylenchus* spp.) in two field trials in the Mpumalanga province (Van Rensburg, 1988). Research on nematicide in maize has been carried out in various studies and experiments across SA, but results showed inconsistencies (Zondagh & Van Rensburg, 1983; McDonald & Nicol, 2005; Bridge & Starr, 2007). Such inconsistencies in nematicide efficacy were mainly due to low or inconsistent rainfall (Mc Donald *et al*., 2017).

1.5.2 Cultural control

Cultural control has been used effectively to reduce the spread and densities of *Meloidogyne* spp. (Moens *et al*., 2009; Karssen *et al*., 2013; Onkendi *et al*., 2014). This technique mainly involves the use of clean planting equipment or/and implements and disinfected fields (Dababat & Fourie, 2018), the use of organic supplements (Thakur, 2014), resistant crops, mixed crops, and crop rotation (Brown *et al*., 2006). Cultural control further supports the development of suppressive soils and viable agricultural practices (Widmer *et al*., 2002).

Crop rotation is a common cultural strategy that controls nematode pests by either planting a non-host, resistant, or poor-host genotype of a given crop (Fourie & De Waele, 2019). Therefore, it is important to consider detailed information about the history of the site, the identity of *Meloidogyne* spp., and its range of hosts before the adoption of the appropriate crop rotation system (Onkendi *et al*., 2014; Fourie *et al*., 2015; Coyne *et al*., 2018). In many instances, however, there are restrictions that may render crop rotation in SA ineffective as a control tool, especially in fields infested with genera that are polyphagous and have a wide host range such as *Meloidogyne* spp. and *Pratylenchus* spp. (Mc Donald *et al*., 2017).

Riekert & Henshaw (1998) highlighted the negative effects of crop rotation on maize rotation (cv. PAN6043) with oilseed crops in sandy soil in the Free State province. This study showed a significant increase (up to 189-fold) of a mixed population of *M. incognita* and *M. javanica* (70:30 ratio) when cowpea (cv. Glenda) and soybean (cv. Knap) were included once during four consecutive planting seasons. In addition, where the initial population densities of *Meloidogyne* spp. was relatively high (> 20,000 roots 50 $g^{-}1$), a 3-fold increase in the number of *Meloidogyne* spp. was observed in the maize and soybean crop rotations. However, according to Bolton & De Waele (1989) maize can be rotated with certain sunflower cultivars to reduce *P. zeae* populations, except in fields where *M. incognita* and *M. javanica* are present.

Soil tillage is another widely used cultural control method, especially in commercial maize growing areas in SA (Du Plessis, 2003). Cabanillas *et al*. (1999), recommended the use of this method in conjunction with crop rotation as a strategy to control PPN. Research has shown that soil tillage reduced pest nematode densities (McDonald & Nicol, 2005), although, other studies proved that tillage had little effect on nematode densities. Repeated tillage in the top 30 cm of the soil layer over a period of 30 days during hot and dry seasons between crops can significantly reduce nematode infestation through drying out of eggs and J2 (Sikora *et al*., 2005). However, this practice is not feasible due to the potential impact of moisture loss after tillage on existing and subsequent crops, especially in SA where dry land production of maize and sunflower dominates.

Cover crop application has been generally executed as another cultural technique which can be used to reduce PPN densities in agricultural soils and are often effective when applied to the soils as manure amendments (Widmer *et al*., 2002). Examples of cover crops that can be used in maize and sunflower-based cropping systems in SA include sundagrass (*Sorghum drummondii*) (Widmer *et al*., 2002), rattlepods (*Crotalaria* spp.) (Wang *et al*., 2002) and *Brassicaceae* (Mojtahedi *et al*., 1991; Lazzeri *et al*., 2004). *Crotalaria* spp. can be adopted as a cover crop to reduce population densities of *Meloidogyne* spp., *Rotylenchulus reniformis* Linford & Oliveira, 1940, *Radopholus similis* (Cobb, 1893) Thorne, 1949, *Belonolaimus longicaudatus* Rau, 1958, and *Heterodera glycines* Ichinohe, 1952. However, this method is often not feasible for crops grown on rainfed fields, as it relies on irrigation (Chitwood, 2002).

1.5.3 Host plant resistance

The use of planting resistant cultivars is not only ecologically safe but also feasible in reducing the population densities of *Meloidogyne* spp. (Boema & Hussey, 1992). Inbred line MP712W from the USA as well as multiple genotypes used by commercial producers and smallholders in SA with proven resistance (Aung *et al*. 1990; Windham and Williams, 1987), were screened against local populations of *M. incognita* race 2 and *M. javanica*. Numerous cultivars, for example DKC8010 and AFG4410, have been shown to be very susceptible to both *Meloidogyne* spp., while others including DKC7815B, PHB3203 and DKC6125B were resistant against one species, but not the other. In this case, these cultivars are not considered an effective strategy as *M. incognita* and *M. javanica* are commonly found in mixed communities in maize and sunflower fields in SA (Kleynhans 1991; Riekert 1996a; Riekert & Henshaw, 1998). As a result, there is no non-host grain cultivars to RKN available in South Africa (Fourie, 2022).

From a global perspective, various studies have been carried out to identify resistant genotypes in maize. For example, in Nigeria, various maize varieties with resistance to *M. incognita* were identified (Adegbite, 2011), while in the USA, a screening for *M. incognita* was carried out in 43 open-pollinated maize varieties in greenhouse tests. The results showed that Tebeau and Old Raccoon consistently shown significant levels of resistance throughout all plantings, with the fewest eggs per gram of fresh root and the lowest reproduction factor (RF) values (0.2 and 0.4, respectively). (222 and 955, respectively). The resistant hybrid resulting from cross Mp307 x Mp707 had the same level of resistance to *M. incognita* as hybrids Bill Dailey and Sheppard Corn, but their resistance levels were less consistent across plantings. The most susceptible hybrids were Levi Mallard, African Bushman, and Field's White Variety, with RF values of 3.1-3.3 and 2,479-3,678 eggs per gram of fresh root, respectively, (Aung *et al*., 1990). Furthermore, Kagoda *et al*. (2011 & 2015) reported maize hybrids with combined resistance against *P. zeae* and *Meloidogyne* spp. Ngobeni *et al*. (2011), reported several open-pollinated hybrids and varieties that are adapted to the climatic conditions of SA and show resistance to *M. incognita* and *M. javanica*.

The only information available for sunflower is that 20 commercially available cultivars,

screened for their host status to *M. incognita*, were identified as being susceptible (Pretorius *et al*., 2014).

1.5.4 Integrated nematode management (INM)

Integrated nematode management (INM) is gaining popularity as old-generation, synthetically derived nematicides are continuously phased out (Sikora & Roberts, 2018). Due to limited financial access, small-scale producers in SSA countries use low-input cultural control techniques such as organic animal and plant manures, crop rotation, and/or intercropping to mitigate *Meloidogyne* spp. damage. However, SA maize and sunflower producers do not use any direct INM technique due to a lack of visible damage and/or insufficient basic information about the effect of *Meloidogyne* spp. on these crops (Fourie, 2022). Commercial producers who are aware of the *Meloidogyne* spp. constraint, produce maize and sunflower with modern agricultural inputs and technologies (Fourie, 2022).

Crucially important as part of an INM strategy, is the presence of weeds that are commonly found in maize and sunflower fields and that are hosts to *Meloidogyne* spp. (Ntidi *et al*., 2012 & 2015). As a result, the build-up of high densities *Meloidogyne* spp. can be prevented by controlling weeds.

Another important aspect is sampling of soil and roots of crops growing actively in a field where maize or sunflower are planned to be grown in the follow-up season (Lamberti & Taylor, 1979) to avoid planting in infested fields. Also, irrigation water around infested fields should not run off and care should be taken to keep equipment clean. Important too is that as a precaution, nematode-infested planting equipment should usually be rinsed with hot water or sprayed with chemicals to kill/inactivate any nematode inoculum and prevent PPN from spreading to non-infested fields (Matlala, 2020).

2. Conclusion

To date, nematode surveys in SA have primarily focused on commercial maize (Mc Donald *et al.,* 2017; Pretorius, 2016) and sunflower (Bolton & De Waele, 1989) fields. Little information and knowledge about nematode pests exist in fields of New-Era producers. For instance, maize yield losses due to PPN assemblages typically dominated by *Meloidogyne* spp. in SA commercial agricultural fields have been assessed to be in the range of 12- 60 % (Mc Donald *et al.,* 2017; Fourie, 2022), whereas no data exist for semi-commercial and commercial New-Era producers in this regard (Mtshali *et al.,* 2002a; Daneel *et al.,* 2003). The same applies for sunflower of which crop losses of 12% have been listed under SA climatic conditions (Keetch, 1989). As a result, emerging maize producers are usually unaware of the damage *Meloidogyne* spp. causes to their crops (Fourie, 2022) and this study was initiated with the focal aim of addressing this gap.

CHAPTER 2: MATERIALS AND METHODS

2.1 Survey

This survey was requested by concerned grain producers, through GrainSA, who experience nematode problems in their fields. Therefore, the 15 fields of such producers were selected for this study.The root and rhizosphere soil samples of two grain crops, *viz*. maize (*Zea mays* L.) and sunflower (*Helianthus annus* L.), were obtained from fields of 15 'New-Era Commercial' grain producers situated in three provinces (Free-State, Gauteng and North-West) of South Africa (SA) during the 2018/19 and 2019/20 growing seasons. (Fig. 2.1).

The representative soil and root samples of crops were obtained during two consecutive time intervals 4 - 6 weeks after planting and 12 weeks after planting. To assess the abundance and incidence of plant-parasitic nematodes (PPN), nine plants were sampled per locality to obtain rhizosphere soil and root samples for extraction, counting and identification purposes. Sampling was randomly done in a zig-zag pattern at each site to ensure that an accurate estimation of nematode populations were recorded (Kleynhans *et al*., 1997; Marais *et al*., 2017). The altitude, climatic conditions and soil physical parameters of the 15 localities sampled are listed in (Table 2.1a &b).

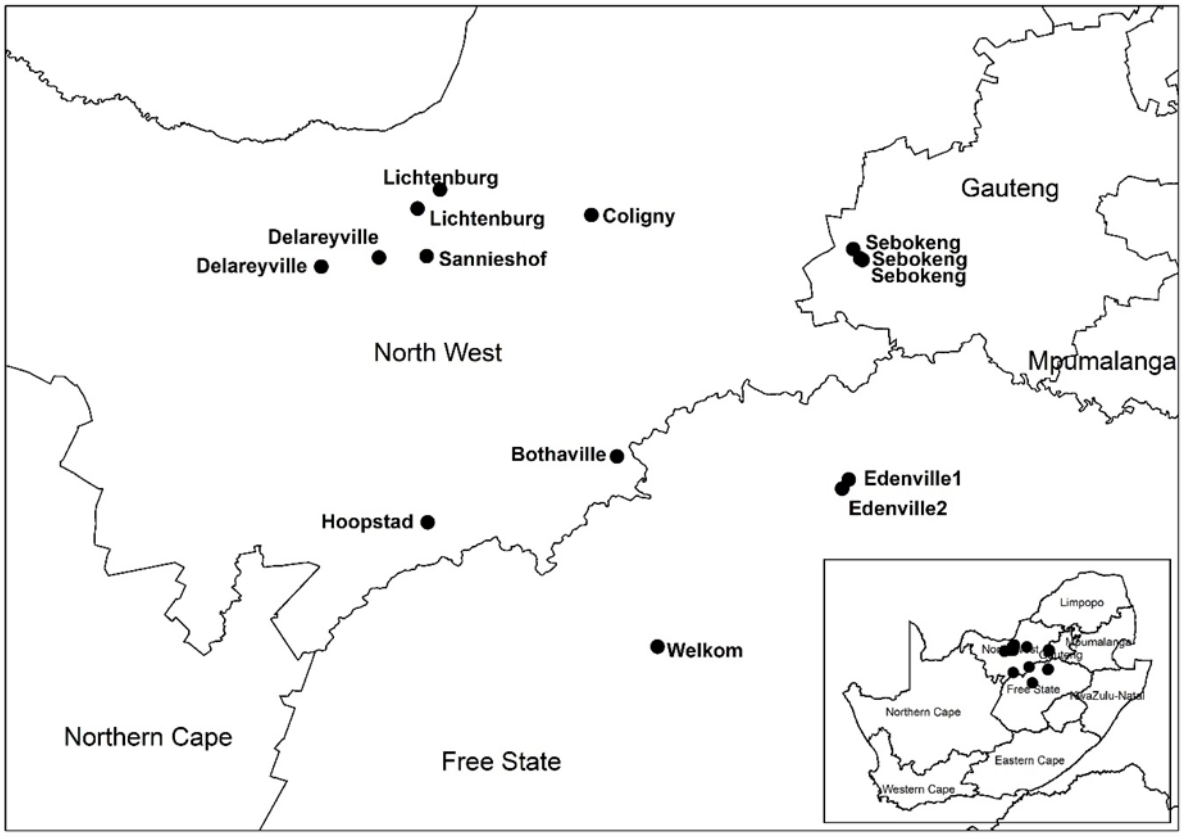


Fig. 2.1 The location of 15 grain fields from which root and soil samples were taken in selected South African maize growing areas for nematode analysis during the 2018/19 and 2019/20 growing seasons.

Table 2.1a The 15 grain fields sampled for a nematode survey during the 2018/19 growing season, with information about the production practices used by the respective producers as well as data about selected soil characteristics.

Localities ID	Farm name	Nearest town	Province	Tillage practice	Previous crop grown (cultivar in parenthesis)	Crop sampled (cultivar in parenthesis)	Nematicide Used	pH (H_2O)	Sand %	Silt %	Clay %
1	Barbespan	Delareyville	North-West	Min tillage	Maize (DKC77-77BR)	Maize (DKC77-77BR & DKC75-66NR)	Metachlor & Terbazyn	4.66	73	4	23
2	Blesbokfotein	Ventersdorp	North-West	Rip	Maize (Pioneer 2432R)	Maize (PANBG5685R)	Roundup; ORTIVIA; JIMBOOST	4.45	71	9	20
3	Bothania	Bothaville	Free-State	Plough	Maize (DKC68-58BR)	Maize (DKC68-58BR)	Roundup	4.52	91	1	8
4	Dwaalkraal	Delareyville	North- West	Rip	Maize (DKC77-77BR)	Maize (DKC77-77BR)	Terbazyn & JUDO SG	5.25	81	3	16
5	Edenville 1	Edenville	Free-State	Rip	Maize (Pioneer 2865BR)	Maize (Pioneer 2865BR)		4.53	77	8	15
6	Edenville 2	Edenville	Free-State	Plough	Maize (DKC68-58BR; DKC68-58BR; DKC75-65BR & DKC76-65BR)	Maize (DKC68-58BR; DKC68-58BR; DKC75-65BR & DKC76-65BR)	Guardian, Mesophlex; Atranex & Magnasuper FC. Roundup; Techniboost; Surebuff & Tyllex	4.55	78	7	15
7	Hoopstad	Hoopstad	Free-State	Min tillage	Maize (PAN 5A-154ZC18)	Maize (PAN 5A-154ZC18)	Terbazyn; STELLERSTAR; Bloodbath for waterpurification	6.63	93	1	6
8	Kaalbass	Sebokeng	Free-State	Plough & rip	MaizeCultivar trial (12 cultivars)	Maize (Pioneer1745, PAN6R-710BR)	Aquaright 5; Touchdown, Primagram; Callisto; Karate	4.64	70	8	22

9	Klipkop 24	Sebokeng	Free-State	Plough & rip	Maize (PAN SA154ZMK)	Maize (PAN SA154ZMK)	Lamgalgo EC; Cheeta, ROUNDUP 700; TOLLAR 840	4.88	59	8	33
10	Klipkob 530	Sebokeng	Free-State	Plough	Maize (PAN5A-154)	Maize (PAN5A-154)	Callisto, Gardmill, Karate, Primagren, Complement	5.41	67	7	26
11	Larestryd	Lichternburg	North-West	Plough	Maize (DKC77-77BR)	Maize (DKC77-77BR)	Metachlor &JUDO SG	6.76	76	5	19
12	Lyshof	Lichternburg	North- West	Plough	Maize (DKC77-77BR)	Maize (DKC77-77BR)	Metachlor	5.51	79	4	17
13	Putfontein	Coligny	North- West	Rip	Maize (Pioneer 2432R)	Maize (PAN2432R)	Roundup, Complement & Poncho	6.54	76	7	17
14	Stella	Delareyville	North-West	Rip	Maize (PAN148)	Maize (PAN148)	Metachlor & Terbazyn	6.76	76	4	9
15	Virginia	Virginia	Free-State	Plough	Maize (Pioneer1745BR ; PAN6R-710BR	Maize (Pioneer1745BR; PAN6R-710BR)	Roundup	5.25	88	5	16

Table 2.1b The 15 grain fields sampled for a nematode survey during the 2019/20 growing season, with information about the production practices used by the respective producers as well as data about selected soil characteristics.

Localities ID	Farm name	Nearest town	Province	Tillage practice	Previous crop grown (cultivar in parenthesis)	Crop sampled (cultivar in parenthesis)	Nematicide Used	pH (H_2O)	Sand %	Silt %	Clay %
1	Barbespan	Delareyville	North-West	Min till	Maize (DKC77-77BR & DKC75-66NR)	Sunflower (AG8251)	Metachlor & Terbazyn	4.64	81	1	18
2	Blesbokfotein	Ventersdorp	North-West	Plough	Maize (PANBG5685R)	Maize (PAN 5785BR)	Roundup; ORTIVIA; JIMBOOST	4.31	75	5	20
3	Bothania	Bothaville	Free-State	Plough	Maize (DKC68-58BR)	Maize (DKC 77-79BR)	Roundup	4.19	92	1	7
4	Dwaalkraal	Delareyville	North-West	Plough & rip	Maize (DKC77-77BR)	Sunflower (AG8251 & 5278)	Terbazyn &JUDO SG	5.65	84	1	15
5	Edenville 1	Edenville	Free-State	Rip	Maize (Pioneer 2865BR)	Maize (Pioneer 2865BR)		4.14	75	5	20
6	Edenville 2	Edenville	Free-State	Plough	Maize (DKC68-58BR; DKC68-58BR; DKC75-65BR & DKC76-65BR)	Maize (DKC73-77BR)	Pre-emer: Guardian, Mesophlex; Atranex & Magnasuper FC. Roundup; Techniboost; Surebuff & Tyllex	4.12	83	3	14
7	Hoopstad	Hoopstad	Free-State	Min till	Maize (PAN SA154ZC18)	Sunflower (PAN7160)	Terbazyn; STELLERSTAR; Bloodbath for waterpurification	6.04	94	1	5
8	Kaalbass	Sebokeng	Free-State	Disc, plough & rip	Maize (Pioneer1745, PAN6R-710BR)	Maize cultivar trial (12 cultivars)	Aquaright 5; Touchdown,	4.16	61	7	32

							Primagram; Callisto; Karate				
9	Klipkop 24	Sebokeng	Free-State	Plough & rip	Maize (PAN SA154ZMK)	Maize (DKC68-58BR)	Lamgalgo EC; Cheeta, ROUNDUP 700; TOLLAR 840	4.20	61	7	32
10	Klipkob 530	Sebokeng	Free-State	Plough	Maize (PAN5A-154))	Maize (PAN 14)	Callisto, Gardmill, Karate, Primagren, Complement	4.83	70	5	25
11	Larestryd	Lichternburg	North-West	Plough	Maize (DKC77-77BR)	Maize (Pioneer R80MK0)	Metachlor &JUDO SG	5.87	77	2	21
12	Lyshof	Lichternburg	North-West	Plough	Maize (DKC77-77BR)	Sunflower (PAN7160CLP)	Metachlor	6.02	79	2	19
13	Putfontein	Coligny	North-West	Rip	Maize (PAN2432R)	Maize (PAN5R691BR)	Roundup, Complement & Poncho	4.44	77	4	19
14	Stella	Delareyville	North-West	Min till	Maize (PAN148)	Maize (PANSC7012KA4)	Metachlor & Terbazyn	5.75	80	2	18
15	Virginia	Virginia	Free-State	Plough	Maize (Pioneer1745BR ; PAN6R-710BR)	Sunflower) (PAN7160CLP	Roundup	4.96	87	1	12

2.1.1 Soil and root sampling for nematode extractions

Nine plants were randomly selected and uprooted, sampling was randomly done in a zig-zag pattern at each site using a garden spade-tool. No composite samples were made and the nine samples per field were used individually for nematode analyses (see Section 2.1.2); this was done to refrain from taking more samples that would have resulted in lower yields and/or income for producers. The above-ground parts of each plant were removed, while the root system together with the attached soil were placed in a labelled plastic bag, representing the rhizosphere soil. The bags were labelled for nematode and soil analyses purposes, respectively, and subsequently sealed, and put in a cooler box to avoid water loss and exposure to high temperatures. The samples were transported to the Agricultural Research Council-Grain Crops Institute (ARC-GCI) laboratory within 24 to 48 h after sampling. Samples were kept in a refrigerator at 5-7 ˚C for a maximum period of five days before nematode extractions, counting and identification were conducted (Marais *et al*., 2017).

2.1.2. Roots (5 g and 50 g) and soil (200 g) processing

Soil and debris adhering to the roots were removed by gently washing the root systems of plants with tap water. From each of the localities, the nine rhizosphere (200 g) soil sub samples as well as nine 5-g and 50-g root samples were used for the extraction of PPN. For soil samples, the decanting and sieving, followed by an adapted sugar-flotation method (Hooper *et al*., 2005) were used for nematode extractions. The sugar centrifugal flotation method (Hooper *et al*., 2005) was used also to extract nematodes from 5 g root samples. An adapted NaOCl method (Riekert, 1995) was used to isolate eggs of sedentary, PPN such as root-knot nematodes (RKN; *Meloidogyne* Göldi, 1887) and second-stage juveniles (J2) from 50 g roots.

2.1.2.1 Roots (5g) nematode extraction

The adapted sugar, centrifugal-flotation method allows the extraction of a wide range of PPN from plant roots (Jenkins, 1964; Hooper *et al*., 2005). A 5 g root sample per plant from each locality sampled, was cut into approximately 2-5 mm fragments, after which it was placed in a 500-ml capacity flask and 250 ml tap water was added to it. The roots were then shredded at medium speed in a domestic kitchen blender for 40

s to release nematodes from the tissues. The solution, containing root pieces and nematodes was rinsed through a 1-mm aperture mesh sieve stacked on a set of 710- and 20-µm-aperture mesh sieves and rinsed with a gentle stream of running tap water. The residue left on the 20-µm-aperture mesh sieve was collected and decanted into 50-ml capacity centrifuge tubes. Next, 2 g of kaolin (supplier and website), a clay mineral that forms a layer and seals off the nematodes at the bottom of the centrifuge tube (Coolen and D'Herde, 1972), was added to each of the tubes and the nematode-water suspensions in tubes were then shaken well, and centrifuged at 1400g (3000 rpm; 14 cm radius) for 5 min.

The supernatant containing mostly debris was decanted and disposed of. Next, amount in ml of sucrose solution with a particular gravity of 1.15 g was added to each tube (containing the nematodes sealed off at the bottom) and shaken to mix thoroughly and to break the kaolin layer, facilitating the nematodes to get into suspension. The tube and its contents was again centrifuged at 2 500 g (4000 rpm; 14 cm radius) for 1 min, mainly to prevent distortion of nematodes from being exposed to sucrose solution for longer (Ingham,1994). The supernatant containing the nematodes was decanted through a 20 µm-aperture mesh sieve and rinsed using a gentle stream of tap water to remove the sucrose solution. Finaly, the nematodes left on the sieve were washed with tap water into a 100 ml plastic container for counting and identification.

2.1.2.3 Roots (50g) nematode and eggs extraction

For the extraction of *Meloidogyne* eggs and J2 from 50 g root samples, the adapted 1% NaOCl-method was used (Riekert, 1995; Hooper *et al*., 2005; Marais *et al.* 2017) since it was specifically developed to extract eggs from sessile, endoparasitic nematode genera such as *Meloidogyne*.

A 50 g (fresh weight) sub sample were obtained per sample from each locality and cut into 10-50 mm pieces and mixed. The root sample was transferred to 400 ml of a 1% NaOCl solution and shaken for approximately 4 min to break down the gelatinous matrix surrounding the eggs. The NaOCl solution containing the J2 and eggs was decanted onto a range of stacked sieves (order from the top to bottom: 250-µm, 75-

µm, 63-µm, 25-µm, and 20-µm aperture mesh). The above-mentioned range of sieves facilitate less clogging on the bottom 20-µm aperture mesh sieve during the extraction process. A vacuum pump (Edwards high vacuum pump, RPM1725/1426, 60/50Hz supplier website) was connected to the 20-µm mesh sieve to apply suction and improve the movement of the solution containing the J2 and eggs through the sieves. Root pieces were rinsed thoroughly with tap water for about 4 min before collecting J2 and eggs on the 25-µm, and 20-µm aperture sieves by washing it into a 100 ml plastic container for counting and identification (Fig. 2.2).

2.1.2.4 Soil (200 g) nematode extraction

Nematodes were extracted from rhizosphere soil samples using the decanting and sieving method, followed by the sugar flotation method (Hooper *et al*., 2005; Marais *et al*., 2017). This methodology enables isolation of the nematode suspension while heavier root and organic material fragments are eliminated. A 200 g rhizosphere soil sample was obtained for each sample and soaked (for a minimum period of 1 h) in a plastic, 1-L capacity container filled with 500 ml tap water. The solution was decanted onto and washed through a 2-mm-aperture mesh sieve into a 5 L plastic bucket. The residue left on the sieve was discarded and the bucket filled up to 5 L with tap water with a stream of tap water and allow to settle for 30 s. The supernatant was decanted onto a 25-µm aperture mesh sieve and the soil remains that settled at the bottom of the bucket was again mixed with a stream of tap water by filling the bucket up. The process was repeated with a 20 s and 10 s, waiting time intervals, respectively. For each of these three soil mixing processes, the supernatant was emptied from the bucket onto a 25-µm aperture mesh sieve. The residue containing the nematodes was left on the 25-µm aperture mesh sieve and washed with distilled water into 50-ml capacity centrifuge tubes. Next, 5 ml kaolin was added to each of the centrifuge tubes, mixed with the nematode suspended and centrifuged at 1 900 g (3 500rpm; 14 cm radius) for 5 min. The supernatant was decanted and discarded from the centrifuge tube in which the nematodes were sealed off at the bottom of the tube. Sixty millilitre of a sucrose solution with a particular gravity of 1.15 g was added to each centrifuge tube and the content of each tubes shaken until the sediment and sucrose solution was mixed. The content of the tubes were centrifuged again at the same speed, 1 900

g (3 500rpm; 14 cm radius), but only for 1 min to prevent distortion of nematodes. The solution containing the nematodes was then rinsed from the tubes onto a 25 µm-aperture mesh sieve and the residue was collected in a 100 ml plastic container for counting and identification purposes.

2.2 Counting and identification of nematode genera

Extracted nematodes obtained from both root and soil samples were identified to genus level using basic morphological features according to the protocols of Heyns (1971) and Kleynhans *et al.* (1996) and counted using a compound microscope (60 – 100x magnification). Briefly elaborate on details such as how counts were conducted, sample size, dilutions and number of aliquotes counted per sample to get the mean, key features to identify the genus.

2.3 Rearing of root-knot nematode species for identification purposes

The eggs and J2 of *Meloidogyne* spp. that were extracted and counted for each locality were inoculated using a 5 ml syringe on roots of tomato seedlings (cvs. Floradade and Moneymaker) that are susceptible to *Meloidogyne* spp. (Fourie *et al*., 2012; Ntidi *et al*., 2019). This way, *Meloidogyne* spp. present at each of the localities sampled were reared *in vivo* in pots in a glasshouse at the ARC-GC to obtain mature females for both morphological and molecular identification (Hartman & Sasser, 1985). The tomato seedlings used for this purpose were planted in individual four-litre capacity plastic pots filled with a methyl bromide-fumigated (1 1620g a.s./2m^3 soil) soil that was also steam-pasteurised for 48 h at 60 °C. This ensured that no nematodes that could have been present in the soil would survive soil and confound the *Meloidogyne* spp. identification. The soil used for this research activity constituted of 4.5% clay, 92.8% sand, 2.4% silt, 0.3% organic matter, and had a pH (H_2O) of 4.35. Ambient temperature ranges of 19±6 °C (minimum) to 26±3 °C (maximum) and a 14L:10D photoperiod were maintained in the glasshouse throughout the 56-day duration of the *Meloidogyne* spp. rearing process. Plants were carefully watered manually (to prevent cross contamination of *Meloidogyne* spp.), by filling up the pot plant saucer three times a week with 200 ml of tap water. Root-knot nematode infected tomato plants with visible root galling were removed from 30 - 56 days after inoculation (DAI) for

molecular and morphological identification of the species present. This period of rearing allowed completion of at least one nematode generation during the growing period of the tomato crops (Kleynhans, 1991; Milne & Du Plessis, 1964; Fourie, 2005).

Mature females of *Meloidogyne* spp. were removed from root galls (present in each tomato root system) with tweezers and a scapel and used for both molecular and morphological identification purposes, as explained in the disscusion below.

2.3.1 Morphological identification of root-knot nematodes

Root-knot nematode infected tomato roots were stained using the sodium-hypochlorite-acid-fuchsin method (Byrd *et al*., 1983) to enable isolation of mature females for further investigation. Staining was performed by placing the root-knot nematode infected root systems of each sample from each field sampled, after having it rinsed with tap water to remove excess soil and particles, into a 1:5 NaOCl:water solution (containing 100 ml NaOCl and 500 ml tap water) solution for 6 min. The root systems of each sample was then rinsed under running tap water for 1 min and placed into 500 ml tap water for 15 min. After 15 min, the root system was rinsed with running tap water for 1 min and immersed into a boiling acid-fuchsin solution (3.5 g acid-fuchsin dissolved in 250 ml acetic acid) for 30 s; this process was conducted in an extraction cabinet.

The root systems of the respective samples per locality were allowed to cool in the acid-fuchsin solution for 30 min at room temperature. After this period, each root system was washed with tap water for 1 min and placed in 10 ml of glycerin. Pink or reddish stained, mature females of *Meloidogyne* spp. were removed from the roots using a scalpel and tweezer (Fig. 2.3). Each female isolated this way was placed in a small drop of 100% lactic acid that was placed on the surface of a plastic Petri dish lid. The body of each female was pierced with a syringe needle and cut behind the neck area using a scalpel or syring needle. The cuticle piece containing the perineal pattern was placed together with the anterior body part of the female body into a drop of glycerin that was placed on a glass microscope slide and thoroughly pressed down to ensure that the outer surface of the posterior part of the cuticle (containing the perineal pattern) was facing upwards. The anterior and posterior parts of the female mounted this way on the same slid were covered with a cover slip and sealed with transparent

nail polish (Cutex). Each mature female of *Meloidogyne* spp. was identified by obeserving the features from protocols described in Table 2.2.

Table 2.2 Description of morphological features (oesophagus and perineal pattern features) of *Meloidogyne enterolobii, M. incognita, and M. javanica.*

***Meloidogyne* species**	***M. enterolobii***	***M. incognita***	***M. javanica***
Perineal pattern features			
General shape	Round to ovoid	Circular to oval	Circular to oval
Dorsal arch	Moderately high to high	Medium high to high	Low to medium
Apex	Rounded, but square in some specimens	Broadly rounded or squared	Squarish to broadly rounded
Phasmids	Distinct phasmids in tail-terminus	Distorted phasmids	No clear, but visible
Tail region	Tail tip terminus visible	Tail tip terminus visible	Tail tip terminus visible
Lateral lines	Not distinct	Not visible	Clearly visible as double line
Striae	Smoother and finer pattern. Non present in peri-vulva area	Smooth, wavy, and even zigzag pattern	Smooth to wavy
Miscellaneous	Perivulvar region free of striae	Whorl around tail terminus	Distinct rectal punctuations
Features of lumen of the oesophagus			
Lumen lining	No information recorded	Expands then narrows towards spheroid	No information
Metacorpus lining	No information recorded	Visible as an ovoid	Spheroid
Procorpus lining	No information recorded	No information	Cylindrical, may expand/narrow immediately in front of usual ovoid

[1]Yang and Eisenback (1983), [2]Eisenback (1985), [3]Kleynhans (1991); [4]Brito *et al*. (2004); [5]Karssen *et al*. (2013); [6]Agenbag (2016); [7]Rashidifard (2019); [8]Collet (2020).

2.4 Biochemical and molecular identification of Meloidogyne spp.

2.4.1 Isolation of females

After the *in vivo* rearing process of *Meloidogyne* spp. populations (containing single species) or communities (containing mixed species) commenced as explained in Section 2.3 above, 10 mature females of *Meloidogyne* spp. were harvested from infected tomato roots representing each of the 15 localities sampled and transferred to individual sterilised 1.5-ml Eppendorf tubes for molecular species identification (see section 2.5.1.1). For biochemical species identification using the isozyme process, one female was removed from infected root tissue and used (see section 2.5.2). This procedure was performed using a stereo microscope (100x magnification). Eppendorf tubes containing the females were refrigerated at 4 °C until DNA extraction commenced for molecular identification.

2.4.1.1 Deoxyribonucleic acid extraction for molecular identification

The 10 females in each tube for each locality sampled were homogenized using a pestle, which was washed and sterilised with a 96% alcohol solution between samples to avoid cross-contamination of female body tissue and vortexed. The DNA extraction procedure followed was adapted by by Rashidifard (2019) from Musapa *et al.* (2013) and was as follows, a 20 µl of a Chelex solution (5% w / v) and 3 µl Proteinase K (20 mg.ml-^{1}) were added to each sterilized Eppendorf tube containing the homogenised female tissue and incubated at 56 °C for 2 h. This process was followed by incubation of the homogenised female tissue for 10 min at 95 °C to allow the release and purification of deoxyribonucleic (DNA) extracts from the females. Subsequently, the homogenised female solution in each tube was centrifuged for 30 s at 11 000 rpm and stored at -20 ˚C until it was used for amplification purposes.

2.4.1.2 Amplification of deoxyribonucleic acid of root-knot nematode females for molecular identification

The sequence characterised amplified region–polymerase chain reaction (SCAR-PCR) procedure described by Rashifard (2019) and Musapa *et al*. (2013) was used to obtain (DNA) bands for each *Meloidogyne* spp. on agarose gel and viewed on an

electrophoresis for identification. The process is described below.

A total volume of 25 µl PCR mix, which contained 18,5 µl (mixture of master mix (Promega Corporation, USA) and ddH2O), 1 µl forward primer (10 µM), 1 µl reverse primer (10 µM), and 4,5 µl of DNA extracted from females of each of the localities samples was added to individual Eppendorf tubes that each represented a locality sampled. The PCR for amplification with species-specific primers of the respective *Meloidogyne* spp. tested for (*M. arenaria*, *M. enterolobii*, *M. incognita* and *M. javanica*) were programmed for 2 min at 94 °C followed by 35 cycles of 30 s at 61 °C, 30 s at the annealing temperature and 1 min at 72 °C. Annealing temperatures were 61°C, 30 s for the Far/Rar primers, 64 °C for Fme /Rme and Fjav/Rjav primers and 64°C for Finc/Rinc primers (Zijlstra *et al*., 2000; Long *et al*., 2006) (Table 2.2b). The prepared Eppendorf tubes were transferred to separate PCR machines that were set according to the conditions required for DNA amplification of each of the four *Meloidogyne* spp. tested. All primer sequences are listed in Table 2.3a. The PCR amplification profiles for each species were applied as listed in Table 2.3b.

2.4.1.3 Gel electrophoresis

A 1% agarose gel solution containing TAE (Tris – Acetate – EDTA) was solidified into the characteristic rectangular shape, used for gel analyses, containing single wells on the upper side in which the PCR products of mature females of *Meloidogyne* spp. each locality sampled were loaded. Before loading these PCR products, a 1 bp ladder was loaded into the first well indicating the band position of each DNA fragment size which would be generated for the *Meloidogyne* spp. under investigation. Each well, after the first one containing the 1 bp ladder, was loaded with a 4 µl droplet of PCR product of the females from the respective localities by drawing it with a micropipette from each of the PCR tubes. Next, 1 µl of GelRed (www.biotium.com) was added to enable staining of the DNA bands so that it will be visible. The same procedure was repeated for each of the four species. The process of separating DNA fragments from the specific *Meloidogyne* spp. using an electric current in the gel (lasting approximately 40 min) was the next step after which the gel was exposed to an ultraviolet transilluminator for inspection of the gel to determine the size of the DNA fragments present. The species standards and the 1 bp ladder were inspected to determine the

size of each of thespecies' DNA bands that were visible on the gel.

Table 2.3a Name, sequence and amplification size of different SCAR primers used for molecular identification of *Meloidogyne* spp.

Target spp.	**Primer name**	**Primer sequence**	**Fragment size**	**References**
M. arenaria	[1]Far	TCG GCG ATA GAG GTA AAT GAC	420 bp	Zijlstra *et al*. (2000)
	[2]Rar	TCG GCG ATA GAC ACT ACA ACT		
M. enterolobii	[1]FMe	AAC TTT TGT GAA AGT GCC GCT	250 bp	Long *et al.* (2006)
	[2]RMe	TCA GTT CAG GCA GGA TCA ACC		
M. incognita	[1]Finc	CTC TGC CCA ATG AGC TGT CC	1200 bp	Zijlstra *et al.* (2000)
	[2]Rinc	CTC TGC CCT CAC ATT AGG		
M. javanica	[1]Fjav	GGT GCG CGA TTG AAC TGA GC	700 bp	Zijlstra *et al.* (2000)
	[2]Rjav	CAG GCC CTT CAG TGG AAC TAT AC		

[1]F=Forward primer, [2]F=Reverse primer

Table 2.3b The profiles for the polymerase chain reaction (PCR) protocols used during this study with different primers for identification of *Meloidogyne* spp. (Zijlstra *et al*., 2000; Long *et al*., 2006).

Primer Pair	Denaturation temperature and time	No of Cycles	Denaturation temperature and time	Annealing temperature and time	Extension temperature and time	Final extension Temperature and time
[1]F-ar/ [2]R-ar	94 ^{0}C, 2 min	35	94 ^{0}C, 30 s	61 ^{0}C, 30 s	72 ^{0}C, 1 min	72 ^{0}C, 5 min
[1]F-me/ [2]R-me	94 ^{0}C, 2 min	35	94 ^{0}C, 30 s	64 ^{0}C, 30 s	72 ^{0}C, 1 min	72 ^{0}C, 5 min
[1]F-inc/ [2]R-inc	94 ^{0}C, 2 min	45	94 ^{0}C, 30 s	54 ^{0}C, 30 s	72 ^{0}C, 1 min	72 ^{0}C, 5 min
[1]F-jav/ [2]R-jav	61 ^{0}C, 2 min	45	94 ^{0}C, 30 s	64 ^{0}C, 30 s	72 ^{0}C, 1 min	72 ^{0}C, 5 min

[1]F = Forward primer, [2]F = Reverse primer, ar = *M. arenaria*, me = *M. entorolobii*, inc = *M. incognita, jav = M.javanica*

3.1 Statistical analyses of nematode data

Nematode data obtained as a result of surveys conducted during the 2018/19 and 2019/20 growing seasons were subjected first to the calculation of prominence value (PV), frequency of occurrence (%OF) and mean population density (MPD) using Excel (Microsoft Windows XP). The PV takes into consideration the population density (PD) as well as frequency of occurrence (%) (De Waele & Jordaan, 1988; Fourie *et al*., 2001) of the 75 relevant nematode family/genus/species.This parameter provides an index to classify the genera / species of nematodes that have been identified during this study. Therefore, high prominence values determine species of nematodes which were predominant in the grain root and soil samples for each locality. By contrast, low PV indicates less important nematode genera / species. The equation used to calculate the PV is as follows (De Waele & Jordaan, 1988).

PV per locality = population density x $\sqrt{}$(frequency of occurrence)/10

with:

- population density per locality = (population density of the genus or species/number of samples in which the genus/species occurred) and
- frequency of occurrence per locality = (number of samples in which a genus occurred/number of samples obtained) x 100.

Population dynamics of *Meloidogyne* and *Rotylenchulus* were investigated over the sampling intervals of the two growing seasons of the survey by submitting the data to Repeated Measures Anova analyses using the software TIBCO Statistica™ Version 14.0.0.15 Software Package (https://www.statistica.com). Moreover, to eludicate potential associations existing between nematodes and selected abiotic factors, principal component analysis (PCA) using ADE4 software (Thioulouse *et al*., 1997) were applied.

CHAPTER 3: RESULTS AND DISCUSSION - NEMATODE SURVEY

3.1 Results

3.11 Nematode data – 50 g roots

Individuals of only two genera, *Meloidogyne* Goeldi, 1892, (root-knot nematodes) and *Rotylenchulus* Linford and Oliveria, 1940 (spiral nematodes) were present in 50 g roots of the grain crops sampled (Tables 3.1 & 3.2). *Meloidogyne* generally dominated with the highest densities recorded being 110344 eggs and J2/50 g roots (2nd sampling during 2020 survey; Table 3.1), while the highest density for *Rotylenchulus* was 6368 eggs and juveniles/50 g roots (2nd sampling 2020 survey; Table 3.2).

3.2.1.1 Meloidogyne

A significant interaction existed for Localities x Sampling intervals indicating that the population densities of *Meloidogyne* spp. eggs and J2 differed significantly for sampling intervals over the localities (Fig. 3.1). For the 15 localities the *Meloidogyne* spp. egg and J2/50 g roots were the highest for the 2nd samplings of localities Hoopstad (110344), followed by Dwaalkraal (38463) where sunflower was grown, and which differed significantly when compared to the other localities (Table A3.1; Fig. 3.1). The lowest *Meloidogyne* spp. eggs and J2/50 g roots were recorded for Virginia's 1st (40) and 2nd (88) samplings of 2019.

Eleven of the 15 localities namely, Blesbokfontein, Bothania, Dwaalkraal, Edenville 1, Hoopstad, Kaalbaas, Klipkop 24, Larestryd, Lyshof, Stella and Virginia showed an increase in *Meloidogyne* spp. egg and J2 numbers/50 g roots over the sampling period, from the 1st sampling of 2019 to the 1st sampling of 2020, with localities, Dwaalkraal and Hoopstad, showing the highest increases of 91% and 95% respectively.

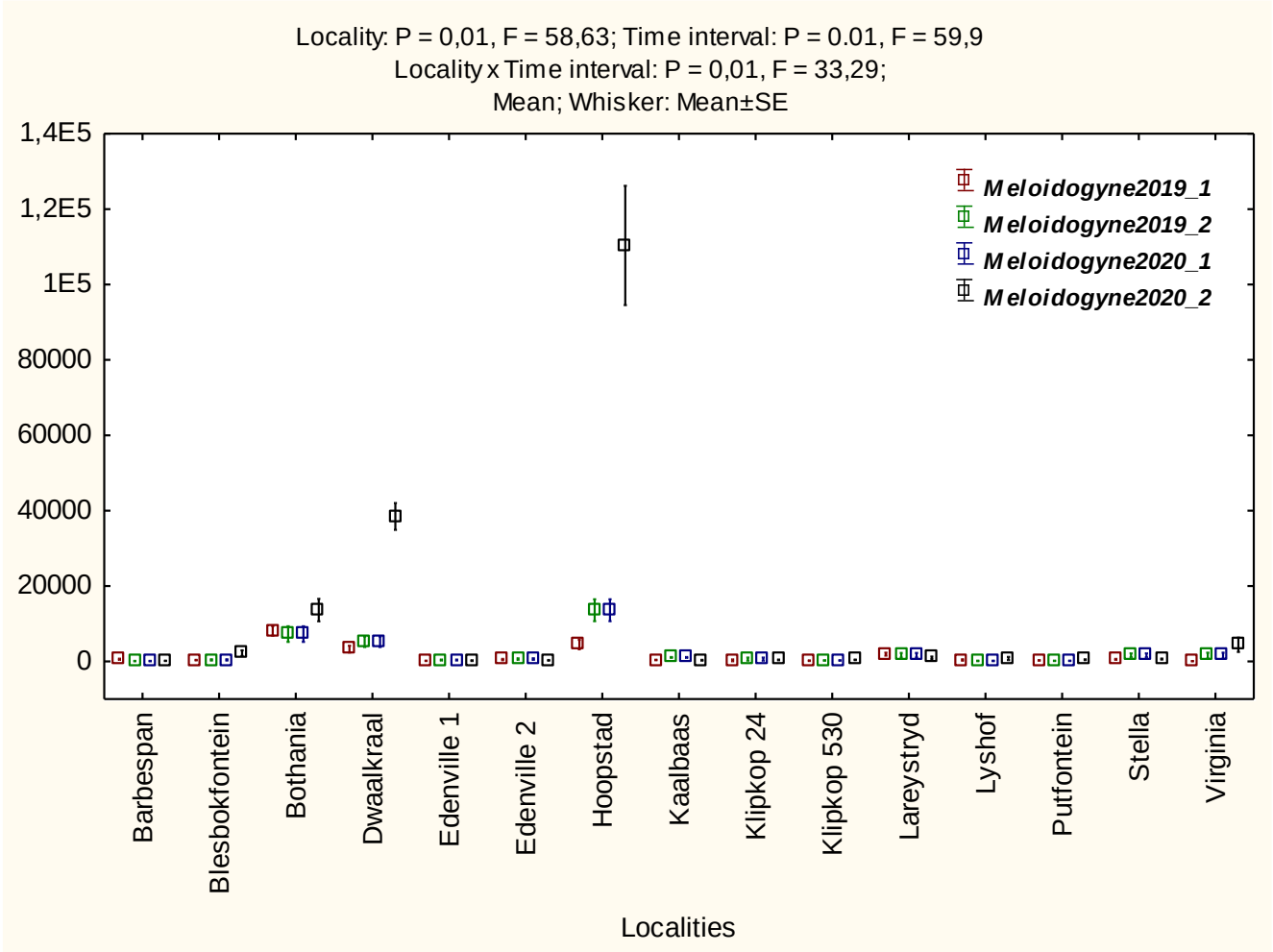


Fig. 3.1 *Meloidogyne* spp. numbers/50 g roots for 15 localities sampled at farms of New-Era grain producers for two seasons (2019 and 2020) and two sampling intervals during each of the seasons. The red arrows show significant differences.

The red arrows indicate that the 2nd sampling intervals of localities Hoopstad and Dwaalkraal had significantly higher *Meloidogyne* spp. abundance compared to the other localities and sampling intervals. The PV for *Meloidogyne* ranged from 100-8099/50 g roots for the 2019 survey's 1st sampling and from 49-6643/50 g roots for the 2nd sampling (Table 3.1). For the 1st sampling of the 2020 survey, the PV for *Meloidogyne* ranged from 120-13586/50 g roots and from 180-110344/50 g roots for the 2nd sampling. The mean population density (MPD) for *Meloidogyne* ranged from 113-8099/50 g roots for the 2019 survey's 1st sampling and from 61-6643/50 g roots for the 2nd sampling; and from 128-13586/ 50 g roots for the 1st sampling of the 2020 survey and 180-110344/50 g roots for the 2nd sampling of the latter growing season (Table 3.1). The frequency of occurrence (FO) for this genus ranged between 67-

100% for the 1st and 2nd sampling of the 2019 survey; and 89-100% for the 1st sampling of the 2020 survey, while it was 100% for the 2nd sampling (Table 3.1).

3.1.1.2 Rotylenchulus

A significant interaction existed for Localities x Sampling intervals indicating that the population densities of *Rotylenchulus* spp. eggs and J2 differed significantly for sampling intervals over the localities (Table A3.2; Fig. 3.2). For the 15 localities the *Rotylenchulus* spp. egg and J2/50g roots were the highest for the 2nd samplings of 2020 for localities Dwaalkraal (6368), followed by Hoopstad (2129) where sunflower was grown (Table A3.1; Fig. 3.1). Dwaalkraal (2nd sampling interval) had significantly higher *Rotylenchulus* spp. egg numbers/50 g roots than all the other localities' sampling intervals, while Hoopstad differed significantly from the following localities' 1st samplings: Barberspan (2020), Dwaalkraal (2020), (Edenville 1 (2019 and 2020), Edenville 2 (2020), Blesbokfontein (2020), Kaalbaas (2019 and 2020), Putfontein (2020), Klipkop 24 (2019 and 2020), Klipkop 530 (2019), Lareystryd (2020), Lyshof (2020), Virginia (2019 and 2020); and the 2nd sampling intervals of Virgina (2019 and 2020), Blesbokfontein (2019), Edenville 1 (2019 and 2020), Barberspan (2019 and 2020), Stella (2019), Kaalbaas (2019), Klipkop 24 (2019), Lyshof (2020), Lareystryd (2019), Putfontein (2020), Edenville 2 (2020), Klipkop 530 (2019 and 2020). The lowest *Rotylenchulus* spp. eggs and J2/50 g roots were recorded for the 2nd samplings of Virginia's 2nd (23) and Blesbokfontein's 2nd (26) samplings of 2019.

Twelve of the 15 localities, namely: Dwaalkraal, Bothania, Edenville 1, Edenville 2, Hoopstad, Kaalbaas, Klipkop 24, Klipkop 530, Lyshof, Putfontein and Virginia showed an increase in *Rotylenchulus* spp. egg and J2 numbers/50 g roots over the sampling period, from the 1st sampling of 2019 to the 2nd sampling of 2022; Dwaalkraal and Hoopstad showed the highest increases of 91 and 69%, respectively (Table A3.1 in Appendix).

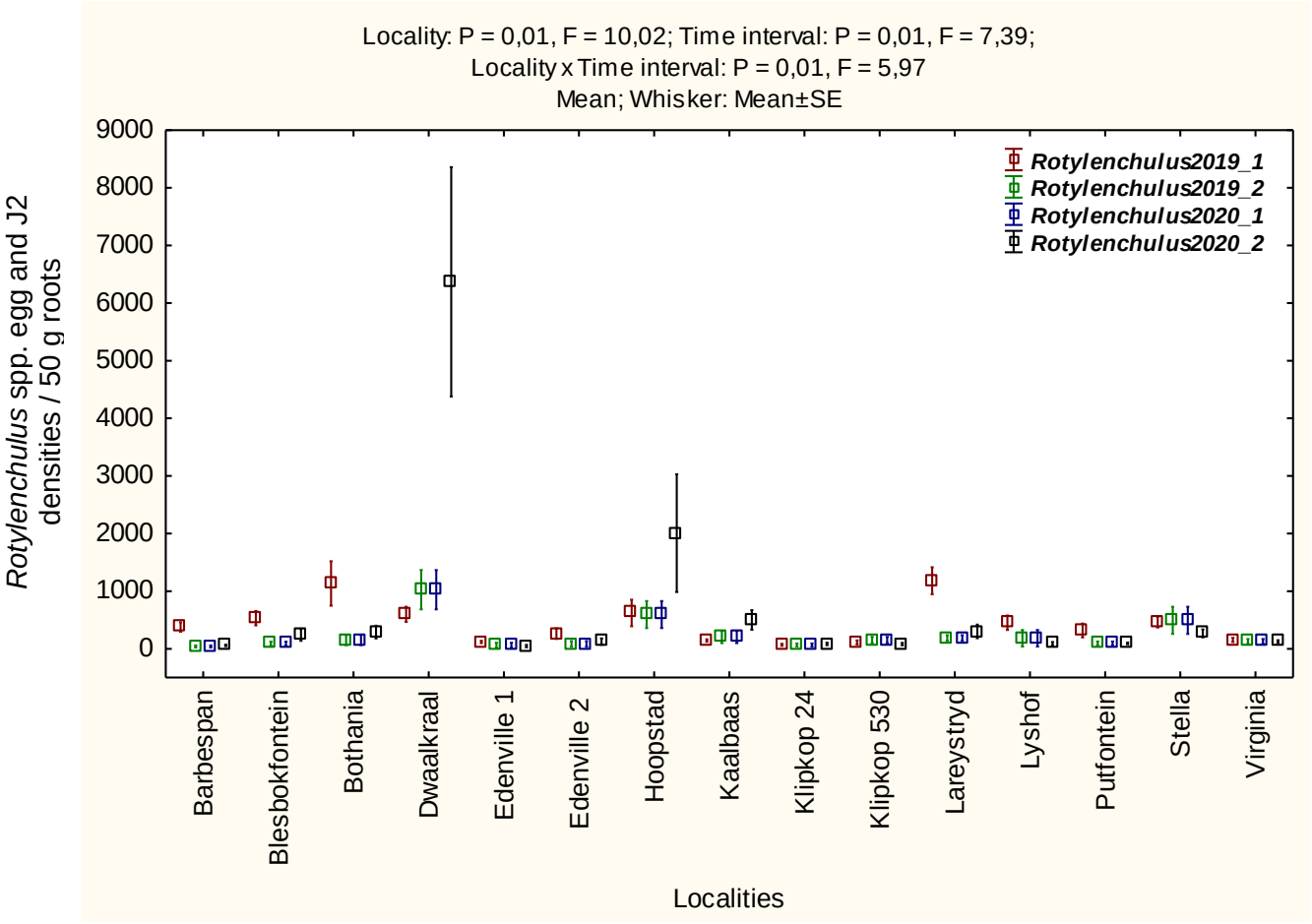


Fig. 3.2 *Rotylenchulus* spp. numbers/50 g roots for 15 localities sampled at farms of New-Era grain producers for two seasons (2019 and 2020) and two sampling intervals during each of the seasons. The red arrows show significant differences.

The PV for *Rotylenchulus* ranged from 78-182/50 g roots for the 2019 survey's 1st sampling and from 28-586/50 g roots for the 2nd sampling; and from 54-970/50 g roots for the 1st sampling of the 2020 survey and 64-6368/50 g roots for the 2nd sampling of the latter growing season (Table 3.2). The MPD for *Rotylenchulus* ranged from 89-1182/50 g the 2019 survey's 1st sampling and from 35-621/50 g roots for the 2nd sampling; and from 73-970/50 g roots for the 1st sampling of the 2020 survey and 86-6368/50 g roots for the 2nd sampling of the latter growing season (Table 3.2).

The FO for this genus ranged from 78-100% for the 1st sampling of the 2019 survey, and from 33-100% in the 2nd sampling; and 33-100% for the 1st sampling of the 2020 survey and 56-100% for the 2nd sampling (Table 3.2).

Table 3.1 Data for *Meloidogyne* numbers/50 g roots for 15 grain fields which were sampled during the 2019/20 crop growing season presented by prominence values (PV), frequency of occurrence (% FO) and mean population densities (MPD) (the data is listed in descending order of PV).

2019 Survey							2020 Survey						
1st sampling				2nd sampling			1st sampling				2nd sampling		
Localities	PV	% FO	MPD	PV	% FO	MPD	**Localities**	PV	% FO	MPD	PV	% FO	MPD
1 (M)	590	100	590	312	100	312	**1 (SF)**	120	89	128	180	100	180
2 (M)	345	100	345	159	89	169	**2 (M)**	392	100	392	2200	100	2200
3 (M)	8099	100	4210	6643	100	6643	**3 (M)**	7269	100	7269	13626	100	13626
4 (M)	3713	78	4210	1646	100	1646	**4 (SF)**	5163	100	5163	38463	100	38463
5 (M)	158	100	158	209	100	209	**5 (M)**	378	100	378	217	100	217
6 (M)	574	78	651	535	100	535	**6 (M)**	683	89	724	299	100	299
7 (M)	4483	100	4483	6385	100	6385	**7(SF)**	13586	100	13586	110344	100	110344
8 (M)	381	100	381	539	100	539	**8 (M)**	1047	100	1047	309	100	309
9 (M)	359	67	440	517	100	517	**9 (M)**	610	100	610	415	100	415
10 (M)	255	100	255	169	100	169	**10 (M)**	257	100	257	427	100	427
11 (M)	1949	100	8099	642	89	681	**11 (M)**	1650	100	1650	975	100	975
12 (M)	442	78	502	400	100	400	**12 (M)**	162	89	172	775	100	775
13 (M)	302	78	343	455	100	455	**13 (M)**	179	89	190	466	100	466
14 (M)	565	100	565	411	100	411	**14 (M)**	1619	100	1619	634	100	634
15 (SF)	100	78	113	49	67	61	**15 (SF)**	1537	100	1537	4413	100	4413

1=Baberspan (North-West), 2=Blesbokfotein (North-West), 3=Bothania (Free-State), 4=Dwaalkraal (North-West), 5= Edenville1 (Free- State), 6=Edenville2 (Free-State), 7=Hoopstad (Free-State), 8=Kaalbaas (Gauteng), 9=Klipkop24 (Gauteng), 10=Klipkop530 Gauteng), 11=Larestryd (North-West), 12=Lyshof (North-West), 13=Putfontein (North-West), 14= Stella (North-West). 15= Virginia (Free-State) *M = Maize; SF = Sunflower.

Table 3.2 Data for *Rotylenchulus* numbers/50 g roots for 15 grain fields which were sampled during 2019/20 crop growing season presented by prominence values (PV), frequency of occurrence (% FO) and mean population densities (MPD) (the data is listed in descending order of PV).

2019 Survey							2020 Survey						
1st sampling				2nd sampling			1st sampling				2nd sampling		
Localities	PV	% FO	MPD	PV	% FO	MPD	**Localities**	PV	% FO	MPD	PV	% FO	MPD
1 (M)	378	100	378	82	89	87	**1 (SF)**	54	56	73	67	78	75
2 (M)	532	100	532	44	33	77	**2 (M)**	100	78	113	258	89	273
3 (M)	1134	100	1134	584	100	584	**3 (M)**	277	56	341	307	89	326
4 (M)	598	100	598	233	89	248	**4 (SF)**	970	100	970	6368	100	6368
5 (M)	126	89	133	63	67	77	**5 (M)**	109	33	189	64	56	86
6 (M)	299	78	339	352	100	352	**6 (M)**	114	56	153	133	89	141
7 (M)	621	100	621	586	89	621	**7 (SF)**	674	78	764	2129	89	2259
8 (M)	143	100	143	81	100	81	**8 (M)**	233	78	264	87	89	92
9 (M)	78	89	89	89	89	95	**9 (M)**	85	56	114	90	89	95
10 (M)	117	89	124	66	89	70	**10 (M)**	153	100	153	500	100	500
11 (M)	1182	100	1182	97	89	103	**11 (M)**	188	100	188	100	78	113
12 (M)	451	100	451	96	78	108	**12 (M)**	275	44	121	301	100	301
13 (M)	318	100	318	341	100	341	**13 (M)**	107	78	494	93	100	93
14 (M)	462	100	462	28	67	35	**14 (M)**	494	100	100	267	100	267
15 (M)	163	89	173	76	64	94	**15 (SF)**	146	78	165	126	100	126

1=Baberspan (North-West), 2=Blesbokfontein (North-West), 3=Bothania (Free-State), 4=Dwaalkraal (North-West), 5= Edenville1 (Free- State), 6=Edenville2 (Free-State), 7=Hoopstad (Free-State), 8=Kaalbaas (Gauteng), 9=Klipkop24 (Gauteng), 10=Klipkop530 (Gauteng), 11=Larestryd (North-West), 12=Lyshof (North-West), 13=Putfontein (North-West), 14= Stella (North-West). 15= Virginia (Free-State) *M = Maize; SF = Sunflower

3.2.2 Nematode data 5 g roots

Plant-parasitic nematodes genera present in 5 g of grain roots during the 2019 and 2020 surveys were *Helicotylenchus, Meloidogyne, Pratylenchus, Rotylenchulus* and *Tylenchorhynchus* (Tables 3.3a, b & c and 3.4a, b & c). The data will be discussed for the predominant genus, followed by those that follow in predominance.

The PV for *Meloidogyne* ranged from 3-64/5 g roots for the 2019 survey's 1st sampling and from 1-48/5 g roots for the 2nd sampling; and from 12-118/5 g roots for the 1st sampling of the 2020 survey and 2-336/5 g roots for the 2nd sampling of the latter growing season (Tables 3.3a & 3.4a). The MPD for *Meloidogyne* ranged from 3-81/5 g roots for the 2019 survey's 1st sampling and from 1-48/5 g roots for the 2nd sampling; and from 13-107/5 g roots for the 1st sampling of the 2020 survey and 2-336/5 g roots for the 2nd sampling of the latter growing season (Tables 3.3b and 3.4b). The FO for this genus ranged from 56-100% for the 1st sampling of the 2019 survey, and from 33-100% for the 2nd sampling; and from 67-100% for the 1st sampling of the 2020 survey and 33-100% for the 2nd sampling (Tables 3.3c and 3.4c).

The PV for *Rotylenchulus* ranged from 0-54/5 g roots for the 1st sampling of the 2019 survey, and from 3-73/5 g roots for the 2nd sampling; and 2-46/5 g roots for the 1st sampling of the 2020 survey and 2-499/5 g roots for the 2nd sampling (Tables 3.3a and 3.4a). The MPD for *Rotylenchulus* ranged from 1-54/5 g roots for the 2019 survey's 1st sampling and from 3-73/5 g roots for the 2nd sampling; and from 2-48/5 g roots for the 1st sampling of the 2020 survey and 2-530/5 g roots for the 2nd sampling of the latter growing season (Tables 3.3b and 3.4b). The FO for this genus ranged from 11-100% for the 1st sampling of the 2019 survey, and from 67-100% in the 2nd sampling; and from 56-100% for the 1st sampling of the 2020 survey and from 67-100% for the 2nd sampling (Tables 3.3c and 3.4c).

Pratylenchus had a PV range of 0 -1/5 g roots for the 1st and 2nd samplings of the 2019 survey, and from 0-3/5 g roots for the 1st sampling of the 2020 survey and 0-5/5 g for the 2nd sampling (Tables 3.3a and 3.4a). The MPD for *Pratylenchus* ranged from 0-1/5g roots for the 2019 survey's 1st sampling and from 0-22/5g roots for the 2nd sampling; and from 0-4/5 g roots for the 1st sampling of the 2020 survey and 0-10/5 g roots for the 2nd sampling of the latter growing season (Tables 3.3b and 3.4b). The FO

for this genus ranged from 0-11% for the 1st sampling of the 2019 survey, and from 0-89% in the 2nd sampling; and 0-44% for the 1st sampling of the 2020 survey and 0-67% for the 2nd sampling (Tables 3.3c and 3.4c).

The PV for *Helicotylenchus* were very low ranging from 0-1/5 g roots for the 1st sampling of the 2019 survey, and from 0-4/5 g roots in the 2nd sampling; and from 0-2/5 g roots for the 1st sampling of the 2020 survey and from 0-14/5 g roots for the 2nd sampling (Tables 3.3a and 3.4a). The MPD for *Helicotylenchus* ranged from 0-2/5 g roots for the 2019 survey's 1st sampling and from 0-11/5g roots for the 2nd sampling; and from 0-4/5 g roots for the 1st sampling of the 2020 survey and 0-21/5 g roots for the 2nd sampling of the latter growing season (Tables 3.3b and 3.4b). The FO for this genus ranged from 0-44% for the 1st sampling of the 2019 survey, and from 0-100% for the 2nd sampling; and from 0-44% for the 1st sampling of the 2020 survey and from 0-100% for the 2nd sampling (Tables 3.3c and 3.4c).

The PV for *Tylenchorhynchus* ranged from 0-1/5 g roots for the 1st and 2nd samplings of the 2019 survey; and 0-2/5 g roots for the 1st and 2nd sampling of the 2020 survey (Tables 3.3a and 3.4a). The MPD for *Tylenchorhynchus* ranged from 0-3/5 g roots for the 2019 survey's 1st sampling and from 0-4/5 g roots for the 2nd sampling; and from 0-4/ 5 g roots for the 1st sampling of the 2020 survey and 0-6/5 g roots for the 2nd sampling of the latter growing season (Tables 3.3b and 3.4b). The FO for this genus ranged from 0-22% for the 1st sampling of the 2019 survey, and from 0-56% in the 2nd sampling; and 0-56% for both the 1st and 2nd sampling of the 2020 survey (Tables 3.3c and 3.4c).

Table 3.3a Prominence values (PV) data for numbers of endo- and semi-endoparasitic nematode genera/5 g roots for 15 grain fields which were sampled during 2019 crop growing season.

	1st sampling					2nd Sampling				
Localities	***Meloidogyne***	***Rotylenchulus***	***Helicotylenchus***	***Pratylenchus***	***Tylenchorhynchus***	***Meloidogyne***	***Rotylenchulus***	***Helicotylenchus***	***Pratylenchus***	***Tylenchorhynchus***
1 (M)	3	1	0	0	0	3	20	0	0	0
2 (M)	3	1	0	0	0	1	4	0	0	1
3 (M)	64	53	0	0	0	2	27	0	0	0
4 (M)	7	4	0	0	0	14	24	2	0	0
5 (M)	27	4	0	0	0	4	32	0	0	0
6 (M)	29	5	0	0	0	48	73	0	0	0
7 (M)	55	48	0	0	0	5	73	0	0	0
8 (M)	14	31	0	0	0	5	9	0	0	0
9 (M)	27	15	0	0	0	13	23	0	0	0
10 (M)	29	54	1	0	0	4	12	0	0	0
11 (M)	14	4	0	1	0	7	33	0	0	0
12 (M)	7	0	0	0	0	13	57	0	0	0
13 (M)	3	1	0	0	0	12	56	0	1	0
14 (M)	11	8	0	0	1	9	38	4	1	0
15 (M)	16	16	0	0	0	1	3	0	0	0

1=Baberspan (North-West), 2=Bleksbokfotein (North-West), 3=Bothania (Free-State), 4=Dwaalkraal (North-West), 5= Edenville1 (Free- State), 6=Edenville2 (Free-State), 7=Hoopstad (Free-State), 8=Kaalbaas (Gauteng), 9=Klipkop24 (Gauteng), 10=Klipkop530 Gauteng), 11=Larestryd (North-West), 12=Lyshof (North-West), 13=Putfontein (North-West), 14=Stella (North-West), 15=Virginia (Free-State). *M = Maize; SF = Sunflower.

Table 3.3b Mean population density (MPD) data for numbers of endo- and semi-endoparasitic nematode genera/5 g roots for 15 grain fields which were sampled during 2019 crop growing season.

	1st sampling					2nd Sampling				
Localities	***Meloidogyne***	***Rotylenchulus***	***Helicotylenchus***	***Pratylenchus***	***Tylenchorhynchus***	***Meloidogyne***	***Rotylenchulus***	***Helicotylenchus***	***Pratylenchus***	***Tylenchorhynchus***
1 (M)	3	3	0	0	0	6	20	1	7	0
2 (M)	4	2	0	0	0	1	5	4	1	3
3 (M)	67	2	0	0	0	3	27	0	0	0
4 (M)	7	5	0	0	0	11	22	7	3	3
5 (M)	29	5	0	0	0	5	22	2	1	0
6 (M)	29	5	0	0	0	48	73	2	3	0
7 (M)	55	48	0	0	0	7	73	3	2	0
8 (M)	14	31	0	0	0	6	9	3	1	0
9 (M)	29	54	0	0	0	13	24	2	2	1
10 (M)	81	15	2	1	0	4	13	1	0	2
11 (M)	14	4	0	0	0	13	57	1	5	2
12 (M)	7	1	0	0	0	8	33	4	2	4
13 (M)	4	3	0	0	0	13	56	11	22	0
14 (M)	71	8	0	0	3	9	38	3	4	2
15 (M)	17	14	0	0	0	2	3	6	1	0

1=Baberspan (North-West), 2=Blesbokfontein (North-West), 3=Bothania (Free-State), 4=Dwaalkraal (North-West), 5= Edenville1 (Free- State), 6=Edenville2 (Free-State), 7=Hoopstad (Free-State), 8=Kaalbaas (Gauteng), 9=Klipkop24 (Gauteng), 10=Klipkop530 Gauteng), 11=Larestryd (North-West), 12=Lyshof (North-West), 13=Putfontein (North-West), 14=Stella (North-West), 15=Virginia (Free-State). *M = Maize; SF = Sunflower.

Table 3.3c Frequency of occurrence (%FO) data for numbers of endo- and semi-endoparasitic nematode genera/5 g roots for 15 grain fields which were sampled during 2019 crop growing season.

	1st sampling					2nd Sampling				
Localities	***Meloidogyne***	***Rotylenchulus***	***Helicotylenchus***	***Pratylenchus***	***Tylenchorhynchus***	***Meloidogyne***	***Rotylenchulus***	***Helicotylenchus***	***Pratylenchus***	***Tylenchorhynchus***
1 (M)	89	11	0	0	0	33	100	23	11	0
2 (M)	56	33	0	0	0	44	78	100	11	33
3 (M)	89	89	0	0	0	67	100	0	0	0
4 (M)	78	56	0	0	0	78	100	56	22	11
5 (M)	89	67	0	0	0	67	100	56	11	0
6 (M)	100	100	0	0	0	100	100	33	11	0
7 (M)	100	100	0	0	0	44	100	22	11	0
8 (M)	100	100	0	0	0	78	100	33	22	0
9 (M)	100	100	0	0	0	100	89	67	22	11
10 (M)	100	100	44	0	0	78	89	11	0	11
11 (M)	100	78	0	11	0	89	100	11	22	11
12 (M)	78	22	0	0	0	100	100	67	89	22
13 (M)	78	22	0	0	0	100	100	11	33	0
14 (M)	56	100	0	0	22	100	100	33	67	56
15 (M)	89	78	0	0	0	33	67	44	22	0

1=Baberspan (North-West), 2=Blesbokfontein (North-West), 3=Bothania (Free-State), 4=Dwaalkraal (North-West), 5= Edenville1 (Free- State), 6=Edenville2 (Free-State), 7=Hoopstad (Free-State), 8=Kaalbaas (Gauteng), 9=Klipkop24 (Gauteng), 10=Klipkop530 Gauteng), 11=Larestryd (North-West), 12=Lyshof (North-West), 13=Putfontein (North-West), 14=Stella (North-West), 15=Virginia (Free-State). *M = Maize; SF = Sunflower.

Table 3.4a Prominence values (PV) data for endo- and semi-endoparasitic nematode genera/5 g roots in 15 grain fields which were sampled during 2020 crop growing season presented.

	1st sampling					2nd Sampling				
Localities	***Meloidogyne***	***Rotylenchulus***	***Helicotylenchus***	***Pratylenchus***	***Tylenchorhynchus***	***Meloidogyne***	***Rotylenchulus***	***Helicotylenchus***	***Pratylenchus***	***Tylenchorhynchus***
1 (SF)	25	9	0	3	0	7	5	1	2	0
2 (M)	118	6	1	0	0	16	20	6	0	1
3 (M)	101	20	0	0	2	134	225	0	0	2
4 (SF)	57	37	1	0	0	336	499	6	5	1
5 (M)	12	20	0	0	0	24	9	2	0	0
6 (M)	28	17	0	0	0	20	5	1	0	0
7 (SF)	105	46	0	0	0	165	80	1	1	0
8 (M)	17	18	0	0	0	7	3	0	0	0
9 (M)	20	20	1	1	1	27	9	1	1	1
10 (M)	13	12	1	0	0	24	23	0	0	1
11 (M)	50	23	2	0	0	38	29	4	2	1
12 (M)	13	2	1	0	1	2	2	2	1	0
13 (M)	20	24	0	0	0	15	10	14	1	1
14 (M)	16	8	2	0	0	13	13	2	4	2
15 (SF)	24	11	1	0	0	13	9	12	0	0

1=Baberspan (North-West), 2=Blesbokfontein (North-West), 3=Bothania (Free-State), 4=Dwaalkraal (North-West), 5= Edenville1 (Free- State), 6=Edenville2 (Free-State), 7=Hoopstad (Free-State), 8=Kaalbaas (Gauteng), 9=Klipkop24 (Gauteng), 10=Klipkop530 Gauteng), 11=Larestryd (North-West), 12=Lyshof (North-West), 13=Putfontein (North-West), 14=Stella (North-West), 15=Virginia (Free-State). *M = Maize; SF = Sunflower.

Table 3.4b Mean population density (MPD) data for numbers of endo- and semi-endoparasitic nematode genera/5 g roots for 15 grain fields which were sampled during 2020 crop growing season.

	1st sampling					2nd Sampling				
Localities	***Meloidogyne***	***Rotylenchulus***	***Helicotylenchus***	***Pratylenchus***	***Tylenchorhynchus***	***Meloidogyne***	***Rotylenchulus***	***Helicotylenchus***	***Pratylenchus***	***Tylenchorhynchus***
1 (SF)	27	10	1	4	1	12	6	1	7	1
2 (M)	22	8	3	1	0	16	20	6	0	3
3 (M)	107	22	0	0	4	142	225	0	0	6
4 (SF)	61	39	2	1	1	336	530	8	10	3
5 (M)	14	25	0	0	0	25	10	3	0	0
6 (M)	28	18	0	0	0	20	5	2	0	0
7 (SF)	105	48	1	0	0	165	80	3	2	0
8 (M)	17	18	0	0	0	8	4	1	0	0
9 (M)	20	20	0	0	2	28	9	2	2	0
10 (M)	13	12	4	4	0	24	23	1	0	3
11 (M)	53	23	0	0	0	38	29	5	4	2
12 (M)	13	2	0	0	1	2	2	5	2	2
13 (M)	21	24	1	1	0	16	10	21	2	1
14 (M)	17	8	2	0	2	15	13	4	4	2
15 (SF)	24	11	2	0	1	14	9	17	3	0

1=Baberspan (North-West), 2=Blesbokfontein (North-West), 3=Bothania (Free-State), 4=Dwaalkraal (North-West), 5= Edenville1 (Free- State), 6=Edenville2 (Free-State), 7=Hoopstad (Free-State), 8=Kaalbaas (Gauteng), 9=Klipkop24 (Gauteng), 10=Klipkop530 Gauteng), 11=Larestryd (North-West), 12=Lyshof (North-West), 13=Putfontein (North-West), 14=Stella (North-West), 15=Virginia (Free-State). *M = Maize; SF = Sunflower.

Table 3.4c Frequency of occurrence (%FO) data for numbers of endo- and semi-endoparasitic nematode genera/5 g roots for 15 grain fields which were sampled during 2020 crop growing season.

	1st sampling					2nd Sampling				
Localities	***Meloidogyne***	***Rotylenchulus***	***Helicotylenchus***	***Pratylenchus***	***Tylenchorhynchus***	***Meloidogyne***	***Rotylenchulus***	***Helicotylenchus***	***Pratylenchus***	***Tylenchorhynchus***
1 (SF)	89	89	11	44	22	33	89	33	11	11
2 (M)	67	67	11	11	0	100	100	100	0	22
3 (M)	89	78	0	0	22	89	100	0	0	11
4 (SF)	89	89	22	11	11	100	89	67	22	11
5 (M)	78	67	0	0	0	89	89	44	0	0
6 (M)	100	89	0	0	0	100	100	22	0	0
7 (SF)	100	89	11	0	0	100	100	22	11	0
8 (M)	100	100	11	0	0	78	67	22	0	0
9 (M)	100	100	0	0	11	89	100	67	33	11
10 (M)	89	100	33	11	0	100	100	11	0	11
11 (M)	89	89	33	0	0	100	100	67	22	11
12 (M)	100	56	22	0	11	67	89	22	11	0
13 (M)	89	78	11	22	11	89	78	44	33	1
14 (M)	89	100	44	0	22	89	67	33	67	56
15 (SF)	100	100	33	0	56	89	89	44	11	0

1=Baberspan (North-West), 2=Blesbokfontein (North-West), 3=Bothania (Free-State), 4=Dwaalkraal (North-West), 5= Edenville1 (Free- State), 6=Edenville2 (Free-State), 7=Hoopstad (Free-State), 8=Kaalbaas (Gauteng), 9=Klipkop24 (Gauteng), 10=Klipkop530 Gauteng), 11=Larestryd (North-West), 12=Lyshof (North-West), 13=Putfontein (North-West), 14=Stella (North-West), 15=Virginia (Free-State). *M = Maize; SF = Sunflower.

3.2.3 Plant-parasitic nematode genera and beneficial nematodes/200 g soil samples

3.2.3.1 Plant-parasitic nematodes

Plant-parasitic nematode genera present in 200 g soil samples of the 2019 survey (Table 3.5a-c) and the 2020 survey (Table 3.6a-c) were *Belonolaimus*, *Crinonema*, *Helicotylenchus*, *Meloidogyne*, *Pratylenchus*, *Rotylenchulus* and *Scutellonema*. The data will be discussed for the predominant genus, followed by those that follow in predominance.

The PV for *Rotylenchulus* ranged from 0-1381/200 g for the 1st sampling of the 2019 survey, and from 0-1122/200 g in the 2nd sampling, and 0-802/200 g for the 1st sampling of the 2020 survey and 0-377/200 g for the 2nd sampling. The MPD for *Rotylenchulus* ranged from 0-1381/200 g for the 2019 survey's 1st sampling and from 0-1122/200 g roots for the 2nd sampling; and from 0-802/200 g for the 1st sampling of the 2020 survey and 0-377/200 g roots for the 2nd sampling of the latter growing season. The FO for this genus ranged from 0-100% for the 1st and 2nd sampling of the 2019 survey, and 1st and 2nd sampling of the 2020 survey.

The PV for *Helicotylenchus* ranged from 0-2270/200 g for the 1st sampling of the 2019 survey, and from 0-47/200 g in the 2nd sampling, and 18-508/200 g for the 1st sampling of the 2020 survey and 6-131/200 g for the 2nd sampling. The MPD for *Helicotylenchus* ranged from 0-2270/200 g for the 2019 survey's 1st sampling and from 0-47/200 g roots for the 2nd sampling; and from 38-539/200 g for the 1st sampling of the 2020 survey and 19-131/200 g for the 2nd sampling of the latter growing season. The FO for this genus ranged from 0-100% for both 1st and 2nd sampling of the 2019 survey, and from 22-100% and 11-100% for both 1st and 2nd sampling of the 2020 survey, respectively.

The PV for *Meloidogyne* and *Pratylenchus* ranged from 0-151/200 g and 0-36/200 g respectively, for the 1st sampling of the 2019 survey, and these genera were not detected in the remaining 3 sampling intervals. The MPD for *Meloidogyne* and *Pratylenchus* ranged from 0-320/200 g and 0-53/200 g respectively, for the 2019 survey's 1st sampling and the latter genera were not detected in the remaining three sampling intervals. The FO *Meloidogyne* and *Pratylenchus* ranged from 0-22% and 0-

11% respectively, for the 1st sampling of the 20219 survey, and both genera were not detected in the remaining 3 sampling intervals.

The PV for *Belonolaimus* ranged from 0-56/200 g for the 1st sampling of the 2019 survey, and from 0-101/200 g in the 2nd sampling, and 0-33/200 g for the 1st sampling of the 2020 survey and 0-18/200 g for the 2nd sampling. The MPD for *Belonolaimus* ranged from 0-171/200 g roots for the 2019 survey's 1st sampling and from 0-356/200 g roots for the 2nd sampling; and from 0-74/200 g roots for the 1st sampling of the 2020 survey and 0-33/200 g roots for the 2nd sampling of the latter growing season. The FO for this genus ranged from 0-56% for the 1st and 2nd sampling of the 2019 survey; and from 0-44% for the 1st sampling of the 2020 survey, and from 0-56% for the 2nd sampling.

The PV for *Criconema* ranged from 0-102/200 g for the 1st sampling of the 2019 survey, and from 0-12/200 g in the 2nd sampling, and 0-75/200 g for the 1st sampling of the 2020 survey and 0-53/200 g for the 2nd sampling. The MPD for *Criconema* ranged from 0-124/200 g roots for the 2019 survey's 1st sampling and from 0-36/200 g roots for the 2nd sampling; and from 0-22/200 g roots for the 1st sampling of the 2020 survey and 0-113/200 g roots for the 2nd sampling of the latter growing season. The FO for this genus ranged from 0-67% for the 1st sampling of the 2019 survey, and from 0-11% in the 2nd sampling; and 0-22% for the 1st and 2nd sampling of the 2020 survey.

The PV for *Scutellonema* ranged from 0-107/200 g for the 1st sampling of the 2019 survey, and from 0-145/200 g in the 2nd sampling and was only detected in the 2nd sampling interval of the 2020 survey (0-4/200 g). The MPD for *Scutellonema* ranged from 0-320/200g roots for the 2019 survey's 1st sampling and from 0-178/200 g roots for the 2nd sampling; and 0-9/200 g roots for the 2nd sampling of the 2020 growing season. The FO for this genus ranged from 0-44% for the 1st sampling of the 2019 survey, and from 0-67% in the 2nd sampling; and 0-22% for the 2nd sampling of the 2022 survey.

Table 3.5a Prominence value (PV) data for endo- and ectoparasitic nematode genera/200 g soil in 15 grain fields which were sampled during 2019 crop growing season presented.

	1st sampling							2nd Sampling						
Localities	***Meloidogyne***	***Pratylenchulus***	***Rotylenchulus***	***Helicotylenchus***	***Scutellonema***	***Criconema***	***Belonolaimus***	***Meloidogyne***	***Pratylenchus***	***Rotylenchulus***	***Helicotylenchus***	***Scutellonema***	***Criconema***	***Belonolaimus***
1 (M)	0	0	191	0	0	102	18	0	0	0	4	3	0	0
2 (M)	0	0	0	0	0	0	0	0	0	0	21	53	0	0
3 (M)	151	0	141	36	62	0	0	0	0	0	0	12	0	47
4 (M)	0	0	36	0	0	0	18	0	0	269	0	24	0	34
5 (M)	0	0	480	133	107	0	0	0	0	1 122	0	145	0	59
6 (M)	0	0	841	32	107	0	11	0	0	5	0	0	0	0
7 (M)	0	18	71	0	0	0	0	0	0	112	12	0	0	13
8 (M)	0	0	0	2 270	22	0	11	0	0	0	47	0	0	0
9 (M)	0	0	1 351	0	0	36	56	0	0	727	5	0	12	27
10 (M)	0	0	1 381	107	0	18	22	0	0	695	7	34	0	0
11 (M)	0	0	189	0	0	0	25	0	0	71	5	67	0	101
12 (M)	0	0	18	87	36	0	0	0	0	0	7	96	0	80
13 (M)	0	0	64	62	80	41	0	0	0	148	4	67	0	34
14 (M)	0	36	261	0	0	0	18	0	0	73	6	0	0	49
15 (M)	0	0	0	0	0	0	0	0	0	436	0	106	12	34

1=Baberspan (North-West), 2=Blesbokfontein (North-West), 3=Bothania (Free-State), 4=Dwaalkraal (North-West), 5= Edenville1 (Free- State), 6=Edenville2 (Free-State), 7=Hoopstad (Free-State), 8=Kaalbaas (Gauteng), 9=Klipkop24 (Gauteng), 10=Klipkop530 Gauteng), 11=Larestryd (North-West), 12=Lyshof (North-West), 13=Putfontein (North-West), 14=Stella (North-West), 15=Virginia (Free-State). *M = Maize; SF = Sunflower.

Table 3.5b Mean population density (MPD) data for endo- and ectoparasitic nematode genera/200 g soil in 15 grain fields which were sampled during 2019 crop growing season presented.

	1st sampling							2nd Sampling						
Localities	***Meloidogyne***	***Pratylenchulus***	***Rotylenchulus***	***Helicotylenchus***	***Scutellonema***	***Criconema***	***Belonolaimus***	***Meloidogyne***	***Pratylenchus***	***Rotylenchulus***	***Helicotylenchus***	***Scutellonema***	***Criconema***	***Belonolaimus***
1 (M)	0	0	0	0	0	0	0	0	0	0	5	71	0	0
2 (M)	0	0	160	53	107	0	0	0	0	0	22	71	0	0
3 (M)	320	0	256	0	0	124	53	0	0	0	1	36	0	71
4 (M)	0	0	107	0	0	0	0	0	0	269	0	71	0	71
5 (M)	0	0	480	200	320	0	107	0	0	462	0	130	36	59
6 (M)	0	0	841	96	71	0	36	0	0	1 122	0	178	0	124
7 (M)	0	53	213	0	0	0	0	0	0	127	36	0	0	356
8 (M)	0	0	0	2 270	101	0	0	0	0	0	47	0	0	0
9 (M)	0	0	1 351	0	0	107	171	0	0	727	10	0	36	47
10 (M)	0	0	1 381	320	0	53	80	0	0	695	12	71	0	0
11 (M)	0	0	231	0	0	0	53	0	0	107	11	142	0	135
12 (M)	0	0	53	117	36	0	0	0	0	0	7	166	0	107
13 (M)	0	0	85	107	120	71	0	0	0	199	5	142	0	71
14 (M)	0	36	320	0	0	0	53	0	0	56	18	0	0	65
15 (M)	0	0	0	0	0	0	0	0	0	0	16	0	0	0

1=Baberspan (North-West), 2=Blesbokfontein (North-West), 3=Bothania (Free-State), 4=Dwaalkraal (North-West), 5= Edenville1 (Free- State), 6=Edenville2 (Free-State), 7=Hoopstad (Free-State), 8=Kaalbaas (Gauteng), 9=Klipkop24 (Gauteng), 10=Klipkop530 Gauteng), 11=Larestryd (North-West), 12=Lyshof (North-West), 13=Putfontein (North-West), 14=Stella (North-West), 15=Virginia (Free-State). *M = Maize; SF = Sunflower.

Table 3.5c Frequency of occurrence (%FO) data for endo- and ectoparasitic nematode genera/200 g soil in 15 grain fields which were sampled during 2019 crop growing season presented.

	1st sampling							2nd Sampling						
Localities	***Meloidogyne***	***Pratylenchulus***	***Rotylenchulus***	***Helicotylenchus***	***Scutellonema***	***Criconema***	***Belonolaimus***	***Meloidogyne***	***Pratylenchus***	***Rotylenchulus***	***Helicotylenchus***	***Scutellonema***	***Criconema***	***Belonolaimus***
1 (M)	0	0	0	0	0	0	0	0	0	0	56	11	0	0
2 (M)	0	0	28	44	33	0	0	0	0	0	89	56	0	0
3 (M)	22	0	56	0	0	67	11	0	0	0	11	11	0	44
4 (M)	0	0	11	0	0	0	0	0	0	100	0	11	0	22
5 (M)	0	0	100	44	11	0	22	0	0	89	0	67	11	33
6 (M)	0	0	100	11	33	0	11	0	0	100	0	67	0	22
7 (M)	0	11	11	0	0	0	0	0	0	78	11	0	0	11
8 (M)	0	0	0	100	22	0	0	0	0	0	100	0	0	0
9 (M)	0	0	100	0	0	11	56	0	0	100	22	0	11	33
10 (M)	0	0	100	11	0	11	22	0	0	100	33	22	0	0
11 (M)	0	0	67	0	0	0	22	0	0	44	22	22	0	56
12 (M)	0	0	11	57	11	0	0	0	0	0	89	33	0	56
13 (M)	0	0	56	53	44	33	0	0	0	56	67	22	0	22
14 (M)	0	11	67	0	0	0	11	0	0	56	56	0	0	56
15 (M)	0	0	0	0	0	0	0	0	0	0	11	0	0	0

1=Baberspan (North-West), 2=Blesbokfontein (North-West), 3=Bothania (Free-State), 4=Dwaalkraal (North-West), 5= Edenville1 (Free- State), 6=Edenville2 (Free-State), 7=Hoopstad (Free-State), 8=Kaalbaas (Gauteng), 9=Klipkop24 (Gauteng), 10=Klipkop530 Gauteng), 11=Larestryd (North-West), 12=Lyshof (North-West), 13=Putfontein (North-West), 14=Stella (North-West), 15=Virginia (Free-State). *M = Maize; SF = Sunflower.

Table 3.6a Prominence value (PV) data for endo- and ectoparasitic nematode genera/200 g soil in 15 grain fields which were sampled during 2020 crop growing season presented.

	1st sampling							2nd Sampling						
Localities	***Meloidogyne***	***Pratylenchulus***	***Rotylenchulus***	***Helicotylenchus***	***Scutellonema***	***Criconema***	***Belonolaimus***	***Meloidogyne***	***Pratylenchus***	***Rotylenchulus***	***Helicotylenchus***	***Scutellonema***	***Criconema***	***Belonolaimus***
1 (SF)	0	0	14	18	0	0	22	0	0	0	9	0	0	3
2 (M)	0	0	802	73	0	0	0	0	0	377	24	0	53	3
3 (M)	0	0	0	31	0	13	13	0	0	0	6	0	0	3
4 (SF)	0	0	6	22	0	0	6	0	0	72	6	0	0	11
5 (M)	0	0	194	78	0	0	6	0	0	34	51	0	0	7
6 (M)	0	0	31	28	0	0	13	0	0	13	32	0	22	0
7 (SF)	0	0	0	18	0	19	0	0	0	0	13	0	0	0
8 (M)	0	0	51	508	0	0	0	0	0	48	131	0	0	3
9 (M)	0	0	250	106	0	75	0	0	0	155	54	0	0	15
10 (M)	0	0	50	82	0	0	22	0	0	152	48	0	6	18
11 (M)	0	0	17	49	0	0	22	0	0	11	58	0	0	13
12 (M)	0	0	0	48	0	19	16	0	0	33	55	3	0	3
13 (M)	0	0	293	86	0	0	13	0	0	70	57	4	7	4
14(M)	0	0	0	75	0	0	33	0	0	0	55	0	0	16
15(SF)	0	0	0	18	0	0	0	0	0	5	9	0	0	0

1=Baberspan (North-West), 2=Blesbokfontein (North-West), 3=Bothania (Free-State), 4=Dwaalkraal (North-West), 5= Edenville1 (Free- State), 6=Edenville2 (Free-State), 7=Hoopstad (Free-State), 8=Kaalbaas (Gauteng), 9=Klipkop24 (Gauteng), 10=Klipkop530 Gauteng), 11=Larestryd (North-West), 12=Lyshof (North-West), 13=Putfontein (North-West), 14=Stella (North-West), 15=Virginia (Free-State). *M = Maize; SF = Sunflower.

Table 3.6b Mean population density (MPD) data for endo- and ectoparasitic nematode genera/200 g soil in 15 grain fields which were sampled during 2020 crop growing season presented.

	1st sampling							2nd Sampling						
Localities	***Meloidogyne***	***Pratylenchulus***	***Rotylenchulus***	***Helicotylenchus***	***Scutellonema***	***Criconema***	***Belonolaimus***	***Meloidogyne***	***Pratylenchus***	***Rotylenchulus***	***Helicotylenchus***	***Scutellonema***	***Criconema***	***Belonolaimus***
1 (SF)	0	0	25	38	0	0	74	0	0	0	19	0	0	9
2 (M)	0	0	802	83	0	0	0	0	0	377	52	0	113	9
3 (M)	0	0	0	41	0	11	28	0	0	0	19	0	0	9
4 (SF)	0	0	19	38	0	0	19	0	0	216	19	0	0	23
5 (M)	0	0	220	117	0	0	19	0	0	59	63	0	0	14
6 (M)	0	0	94	42	0	0	38	0	0	38	36	0	66	0
7 (SF)	0	0	0	38	0	11	0	0	0	0	38	0	0	0
8 (M)	0	0	88	539	0	0	0	0	0	73	131	0	0	9
9 (M)	0	0	265	143	0	22	0	0	0	155	62	0	0	33
10 (M)	0	0	75	100	0	0	38	0	0	162	73	0	12	24
11 (M)	0	0	23	52	0	0	33	0	0	23	66	0	0	22
12 (M)	0	0	0	64	0	11	23	0	0	70	59	9	0	9
13 (M)	0	0	332	91	0	0	13	0	0	79	70	9	14	9
14 (M)	0	0	0	79	0	0	41	0	0	0	73	0	0	23
15 (SF)	0	0	0	38	0	0	0	0	0	9	28	0	0	0

1=Baberspan (North-West), 2=Blesbokfontein (North-West), 3=Bothania (Free-State), 4=Dwaalkraal (North-West), 5= Edenville1 (Free- State), 6=Edenville2 (Free-State), 7=Hoopstad (Free-State), 8=Kaalbaas (Gauteng), 9=Klipkop24 (Gauteng), 10=Klipkop530 Gauteng), 11=Larestryd (North-West), 12=Lyshof (North-West), 13=Putfontein (North-West), 14=Stella (North-West), 15=Virginia (Free-State). *M = Maize; SF = Sunflower.

Table 3.6c Frequency of occurrence (%FO) data for endo- and ectoparasitic nematode genera/200 g soil in 15 grain fields which were sampled during 2020 crop growing season presented.

	1st sampling							2nd Sampling						
Localities	***Meloidogyne***	***Pratylenchulus***	***Rotylenchulus***	***Helicotylenchus***	***Scutellonema***	***Criconema***	***Belonolaimus***	***Meloidogyne***	***Pratylenchus***	***Rotylenchulus***	***Helicotylenchus***	***Scutellonema***	***Criconema***	***Belonolaimus***
1 (SF)	0	0	33	22	0	0	22	0	0	0	22	0	0	11
2 (M)	0	0	100	78	0	0	0	0	0	100	22	0	22	11
3 (M)	0	0	0	56	0	11	22	0	0	0	11	0	0	11
4 (SF)	0	0	11	33	0	0	11	0	0	11	11	0	0	22
5 (M)	0	0	78	44	0	0	11	0	0	33	67	0	0	22
6 (M)	0	0	11	44	0	0	11	0	0	11	78	0	11	0
7 (SF)	0	0	0	22	0	11	0	0	0	0	11	0	0	0
8 (M)	0	0	33	89	0	0	0	0	0	44	100	0	0	11
9 (M)	0	0	89	56	0	22	0	0	0	100	78	0	0	22
10 (M)	0	0	44	67	0	0	33	0	0	89	44	0	22	56
11 (M)	0	0	56	89	0	0	44	0	0	22	78	0	0	33
12 (M)	0	0	0	56	0	11	44	0	0	22	89	11	0	11
13 (M)	0	0	78	89	0	0	11	0	0	78	67	22	22	22
14 (M)	0	0	0	100	0	0	6	0	0	0	73	0	0	44
15 (SF)	0	0	0	22	0	0	0	0	0	33	28	0	0	0

1=Baberspan (North-West), 2=Blesbokfontein (North-West), 3=Bothania (Free-State), 4=Dwaalkraal (North-West), 5= Edenville1 (Free- State), 6=Edenville2 (Free-State), 7=Hoopstad (Free-State), 8=Kaalbaas (Gauteng), 9=Klipkop24 (Gauteng), 10=Klipkop530 Gauteng), 11=Larestryd (North-West), 12=Lyshof (North-West), 13=Putfontein (North-West), 14=Stella (North-West), 15=Virginia (Free-State). *M = Maize; SF = Sunflower.

3.2.3.1 Beneficial nematodes

The trophic groups of beneficial nematodes included bacterivores, fungivores and omnivores and predators in 200 g soil samples collected during 2019 and 2020 surveys (Tables 3.7a, b & c). The data will be discussed for the predominant trophic group, followed by those that follow in predominance.

The PV for bacterivores/200 g soil ranged from 126-782 for the 1st sampling of the 2019 survey, and from 125-1039 in the 2nd sampling, and 51-183 for the 1st sampling of the 2020 survey and 22-456 for the 2nd sampling. The MPD for bacterivores ranged from133-782 for the 2019 survey's 1st sampling and from 125-1039 for the 2nd sampling; and from 54-192 for the 1st sampling of the 2020 survey and 25-106 for the 2nd sampling of the latter growing season. The FO for these nematodes ranged from 89-100% for the 1st sampling of the 2019 survey, and from 78-100% in the 2nd sampling; and 67-100% for the 1st sampling of the 2020 survey, and 56-100% for the 2nd sampling.

The PV for fungivores/200 g soil ranged from 36-588 for the 1st sampling of the 2019 survey, and from 34-244 in the 2nd sampling, and 5-66 for the 1st sampling of the 2020 survey and 4-429 for the 2nd sampling. The MPD for fungivores ranged from 71-789 for the 2019 survey's 1st sampling and from 57-327 for the 2nd sampling; and from 11-75 for the 1st sampling of the 2020 survey and 9-455 for the 2nd sampling of the latter growing season. The FO for these nematodes ranged from 11-78% for the 1st sampling of the 2019 survey, and from 22-89%in the 2nd sampling; and 22-89% for the 1st sampling of the 2020 survey, and 22-89% for the 2nd sampling.

The PV for omnivore and predators/200 g soil ranged from 0-38 for the 1st sampling of the 2019 survey, from 0-36 in the 2nd sampling, and 0-32 for the 1st sampling of the 2020 survey and 0-14 for the 2nd sampling. The MPD for omnivore and predators ranged from 0-107 roots for both 1st and 2nd sampling for the 2019 survey's; and from 0-56 for the 1st sampling of the 2020 survey and 0-25 for the 2nd sampling of the latter growing season. The FO for these nematodes ranged from 0-33% for the 1st sampling of the 2019 survey, and from 0-44% in the 2nd sampling; and 0-78% for the 1st sampling of the 2020 survey, and 0-56% for the 2nd sampling.

Table 3.7a Data for beneficial nematode numbers/ 200 g soil for 15 grain fields which were sampled during 2019 and 2020 crop growing seasons presented by prominence values (PV).

2019 Survey							2020 Survey						
	1st sampling			2nd sampling				1st sampling			2nd sampling		
Localities	Bacterivores	Fungivores	Omnivores & predators	Bacterivores	Fungivores	Omnivores & predators	Localities	Bacterivores	Fungivores	Omnivores & predators	Bacterivores	Fungivores	Omnivores & predators
1 (M)	126	36	0	312	42	12	**1 (SF)**	77	25	19	22	4	5
2 (M)	314	53	18	541	197	36	**2 (M)**	110	36	32	36	17	7
3 (M)	782	215	0	253	63	0	**3 (M)**	86	25	25	456	57	9
4 (M)	279	41	0	591	205	12	**4 (SF)**	106	18	0	25	101	4
5 (M)	492	111	0	379	122	24	**5 (M)**	161	66	25	36	11	7
6 (M)	563	72	0	125	150	0	**6 (M)**	74	28	6	93	32	5
7 (M)	258	588	0	130	119	0	**7 (SF)**	51	43	13	28	429	5
8 (M)	459	195	38	269	41	21	**8 (M)**	112	51	19	25	5	10
9 (M)	492	123	18	565	34	36	**9 (M)**	156	40	22	31	7	0
10 (M)	551	134	25	383	96	12	**10 (M)**	152	44	9	46	4	3
11 (M)	373	56	0	1039	244	12	**11 (M)**	167	41	18	36	18	11
12 (M)	458	196	31	893	134	25	**12 (M)**	106	5	22	64	18	9
13 (M)	184	74	0	371	87	12	**13 (M)**	181	34	28	77	38	14
14 (M)	327	62	36	325	101	0	**14 (M)**	183	47	0	42	4	3
15 (M)	327	64	25	180	59	0	**15(SF)**	116	18	26	46	38	3

1=Baberspan (North-West), 2=Blesbokfontein (North-West), 3=Bothania (Free-State), 4=Dwaalkraal (North-West), 5= Edenville1 (Free- State), 6=Edenville2 (Free-State), 7=Hoopstad (Free-State), 8=Kaalbaas (Gauteng), 9=Klipkop24 (Gauteng), 10=Klipkop530 Gauteng), 11=Larestryd (North-West), 12=Lusthof (North-West), 13=Putfontein (North-West), 14=Stella (North-West), 15=Virginia (Free-State). *M = Maize; SF = Sunflower.

Table 3.7b Data for beneficial nematode numbers soil for 15 grain fields which were sampled during 2019/20 crop growing season presented by mean population density (MPD).

2019 Survey							2020 Survey						
	1st sampling			2nd sampling				1st sampling			2nd sampling		
Localities	Bacterivores	Fungivores	Omnivores & predators	Bacterivores	Fungivores	Omnivores & predators	Localities	Bacterivores	Fungivores	Omnivores & predators	Bacterivores	Fungivores	Omnivores & predators
1 (M)	133	107	0	312	57	36	**1 (SF)**	82	34	56	25	9	9
2 (M)	314	160	53	541	209	53	**2 (M)**	117	49	56	49	20	14
3 (M)	782	244	0	253	71	0	**3 (M)**	91	34	38	60	77	19
4 (M)	279	71	0	627	218	36	**4 (SF)**	113	31	0	34	123	9
5 (M)	492	149	0	191	129	71	**5 (M)**	171	75	34	49	19	14
6 (M)	563	96	0	379	184	0	**6 (M)**	91	42	19	106	34	9
7 (M)	273	789	0	138	178	0	**7 (SF)**	54	53	28	34	455	9
8 (M)	487	221	80	269	71	36	**8 (M)**	138	63	28	38	9	13
9 (M)	492	151	53	565	71	107	**9 (M)**	156	42	33	35	13	0
10 (M)	551	152	25	383	166	36	**10 (M)**	162	61	19	49	9	9
11 (M)	373	78	0	1039	327	36	**11 (M)**	167	61	31	49	31	16
12 (M)	488	240	53	893	152	53	**12 (M)**	113	11	33	64	20	14
13 (M)	184	84	0	371	107	36	**13 (M)**	192	34	32	87	51	25
14 (M)	347	107	107	325	151	0	**14 (M)**	183	47	0	45	9	9
15 (M)	347	85	53	125	89	0	**15(SF)**	131	18	31	46	56	9

1=Baberspan (North-West), 2=Blesbokfontein (North-West), 3=Bothania (Free-State), 4=Dwaalkraal (North-West), 5= Edenville1 (Free- State), 6=Edenville2 (Free-State), 7=Hoopstad (Free-State), 8=Kaalbaas (Gauteng), 9=Klipkop24 (Gauteng), 10=Klipkop530 Gauteng), 11=Larestryd (North-West), 12=Lyshof (North-West), 13=Putfontein (North-West), 14=Stella (North-West), 15=Virginia (Free-State). *M = Maize; SF = Sunflower.

Table 3.7c Data for beneficial nematode numbers/200 g soil for 15 grain fields which were sampled during 2019/20 crop growing season presented by frequency of occurrence (%OF).

2019 Survey							2020 Survey						
	1st sampling			2nd sampling				1st sampling			2nd sampling		
Localities	Bacterivores	Fungivores	Omnivores & predators	Bacterivores	Fungivores	Omnivores & predators	Localities	Bacterivores	Fungivores	Omnivores & predators	Bacterivores	Fungivores	Omnivores & predators
1 (M)	89	11	0	**100**	56	11	**1 (SF)**	89	56	11	78	22	33
2 (M)	100	11	11	**100**	89	44	**2 (M)**	89	56	33	56	67	22
3 (M)	100	78	0	**100**	78	0	**3 (M)**	89	56	44	89	56	22
4 (M)	100	33	0	**89**	89	11	**4 (SF)**	89	33	0	56	67	22
5 (M)	100	56	0	**89**	89	11	**5 (M)**	89	78	56	56	33	22
6 (M)	100	56	0	**100**	67	0	**6 (M)**	67	44	11	78	89	33
7 (M)	89	56	0	**89**	44	0	**7 (SF)**	89	67	22	67	89	33
8 (M)	89	78	22	**100**	33	33	**8 (M)**	67	67	11	44	33	56
9 (M)	100	67	11	**100**	22	11	**9 (M)**	100	89	44	78	33	0
10 (M)	100	78	22	**100**	33	11	**10 (M)**	89	44	22	89	22	11
11 (M)	100	56	0	**100**	56	11	**11 (M)**	100	44	33	56	33	44
12 (M)	100	67	33	**100**	78	22	**12 (M)**	89	22	44	100	78	44
13 (M)	100	78	0	**100**	67	11	**13 (M)**	89	44	78	78	56	33
14 (M)	89	33	11	**89**	44	0	**14 (M)**	100	78	0	89	22	11
15 (M)	89	56	22	**78**	44	0	**15 (SF)**	78	33	67	100	44	11

1=Baberspan (North-West), 2=Blesbokfontein (North-West), 3=Bothania (Free-State), 4=Dwaalkraal (North-West), 5= Edenville1 (Free- State), 6=Edenville2 (Free-State), 7=Hoopstad (Free-State), 8=Kaalbaas (Gauteng), 9=Klipkop24 (Gauteng), 10=Klipkop530 Gauteng), 11=Larestryd (North-West), 12=Lyshof (North-West), 13=Putfontein (North-West), 14=Stella (North-West), 15=Virginia (Free-State). *M = Maize; SF = Sunflower.

3.3 Associations between nematodes and selected abiotic parameters

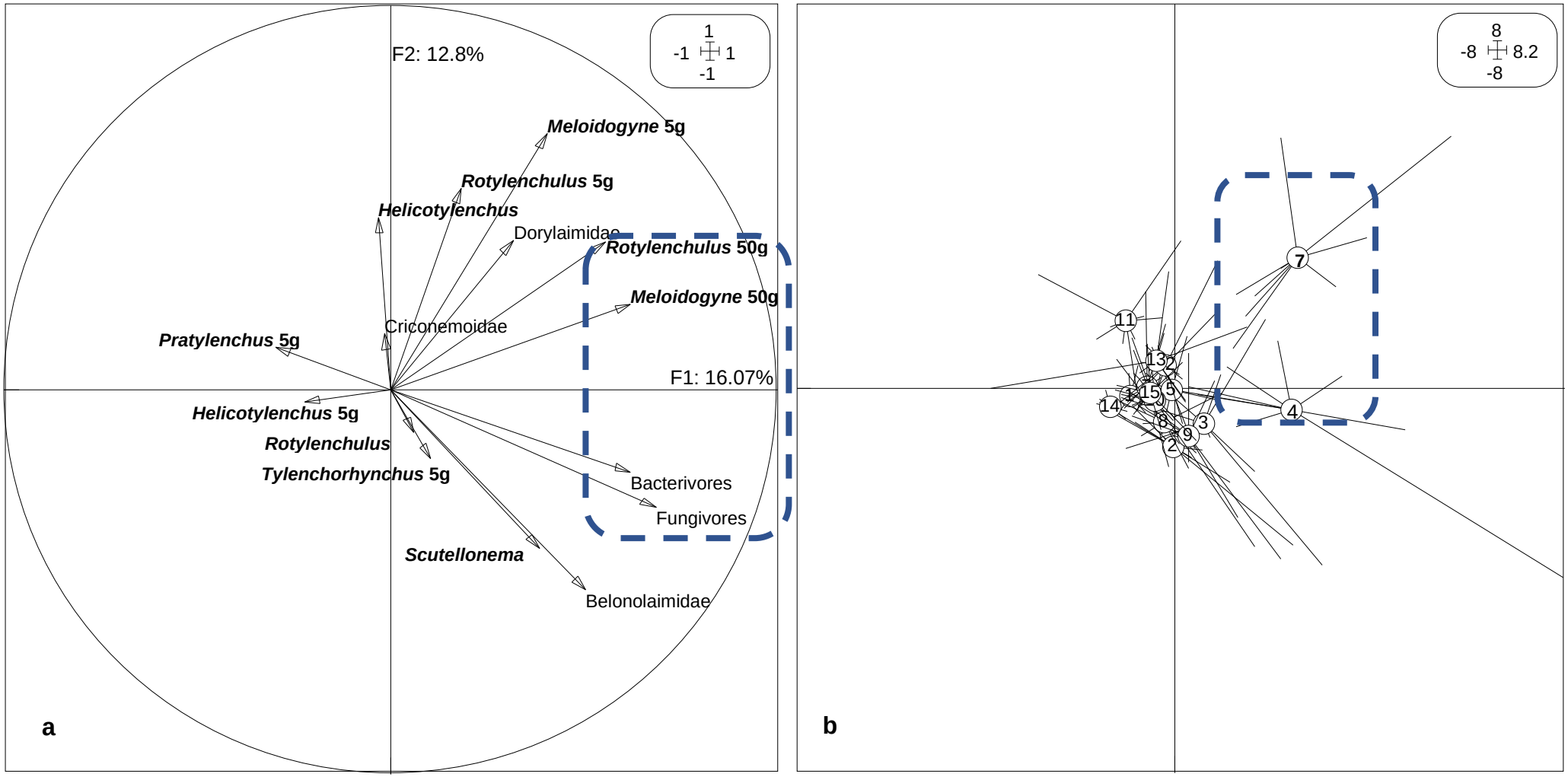
The following associations were investigated: Nematodes (PPN and beneficial) x Locations; and Nematodes (PPN and beneficial) x Soil parameters [Ammonium (N-NH4), P(Bray-1,mg/kg), carbon (%C), calcium (Ca, mg/kg), %clay, potassium (K, mg/kg), magnesium (Mg, mg/kg), pH, phosphorus (P, mg/kg), resistance (ohms), %sand, %silt, sodium (Na, mg/kg) and Zinc (Z, mg/kg)]. Furthermore, only data for the 2nd sampling for both the 2019 and 2020 surveys were used for PCA since it represents the time nearest to the end of the growing season. These observations can provide valuable information in terms of planning of management strategies (for the follow-up growing season) to mitigate nematode damage in the fields of producers.

For the 2019 survey's 2nd sampling, the first two factors of the PCA described a low 28.9% of the variation for the nematode data, which represents a weak structure (Fig 3.3a). *Meloidogyne* and *Rotylenchulus* abundance/50 g roots, as well as bacterivores and fungivores/200 g soil had the highest correlation on the positive side of the F1. When the nematode data were projected on the F1 x F2 factorial plane according to locations sampled, locations 4 (Dwaalkraal, North West province) and 7 (Hoopstad, Free-State province) were positioned in the positive part of F1 and were suggested to have the highest *Meloidogyne* and *Rotylenchulus* abundance/50 g roots for these two predominantly occurring PPN (Fig.3.3a and b). A similar trend was observed for the same two locations (4: Dwaalkraal, North West province and 7: Hoopstad, Free-State province) with regards to bacterivores and fungivores/200 g soil. The other locations clustered together and showed no association with specific PPN or beneficial nematodes.

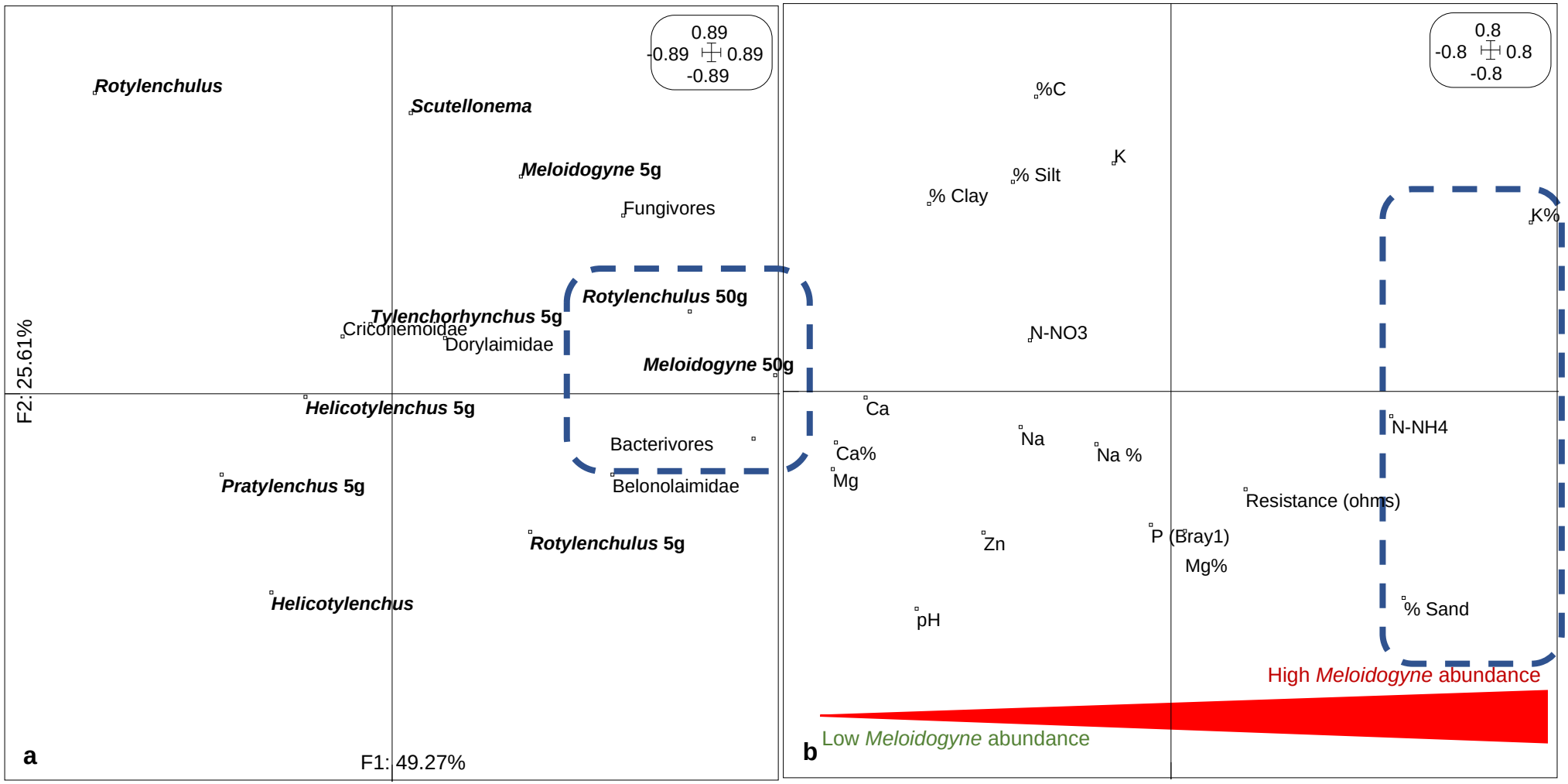
The factorial map for the co-inertia analysis of the 2019 survey's data (2nd sampling) showed that *Meloidogyne* and *Rotylenchulus* abundance/50 g roots, as well as bacterivore abundance/200 g soil were strongly correlated to the positive values of F1 (Fig 3.4a). For the localities, the factorial map showed that %Sand, K (mg/kg) and N-NH4(mg/kg) were strongly correlated to the positive values of F1 (Fig. 3.4b) indicating that higher values of these soil parameters were associated with higher abundance of *Meloidogyne* and *Rotylenchulus*/50 g roots, and higher abundance of bacterivores/200 g soil.

For the 2nd sampling of the 2020 survey, the first two factors of the PCA described a low 29.6% of the variation for the nematode data, representing a weak structure (Fig. 3.5a) as was evident for the 2019 data above. The *Meloidogyne* and *Rotylenchulus* abundance/50 g roots, as well as the abundance of fungivores/200 g soil plotted in the positive side of F2 and had the highest correlation. Projection of the nematode data on the F1 x F2 factorial plane according to locations sampled, showed that locations 4 (Dwaalkraal, North-West province) and 7 (Hoopstad, Free-State province) plotted on the positive side of F2 and were suggested to have the highest *Meloidogyne* and *Rotylenchulus* abundance/50 g roots and fungivore abundance/200 g soil (Fig.3.5b). The other locations clustered together and showed no association with specific PPN or beneficial nematodes.

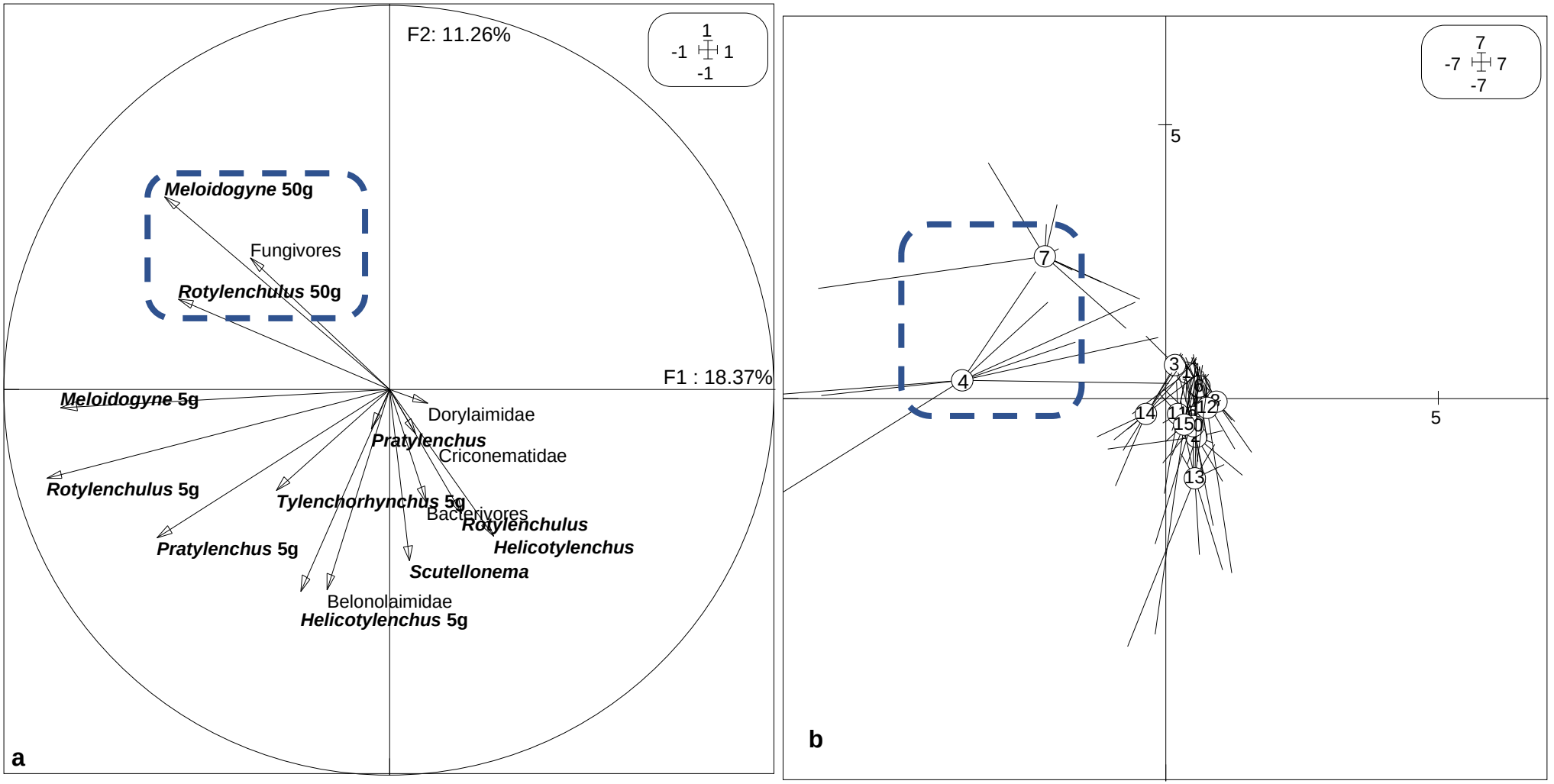
For the 2nd sampling of the 2020 survey, the factorial map for co-inertia analysis indicates that *Meloidogyne* abundance/50 g roots and fungivores/200 g soil plotted on the negative side of the F2 and strongly correlated with such F2 values (Fig. 3.6a). Concerning the localities, the soil parameters %Sand and pH also strongly correlated with negative values of F2 suggesting that high *Meloidogyne* abundance/50 g roots and high fungivore abundance/200 g soil occurred where %Sand and pH values were high (Fig. 3.6b). The opposite was evident for %Clay, %Silt and %C, which plotted and correlated with F1 values and hence showed negatively correlations with *Meloidogyne* abundance/50 g roots and fungivores/200 g soil (meaning that low numbers of these nematodes were present at localities that had high %Clay, %Silt and %C values).



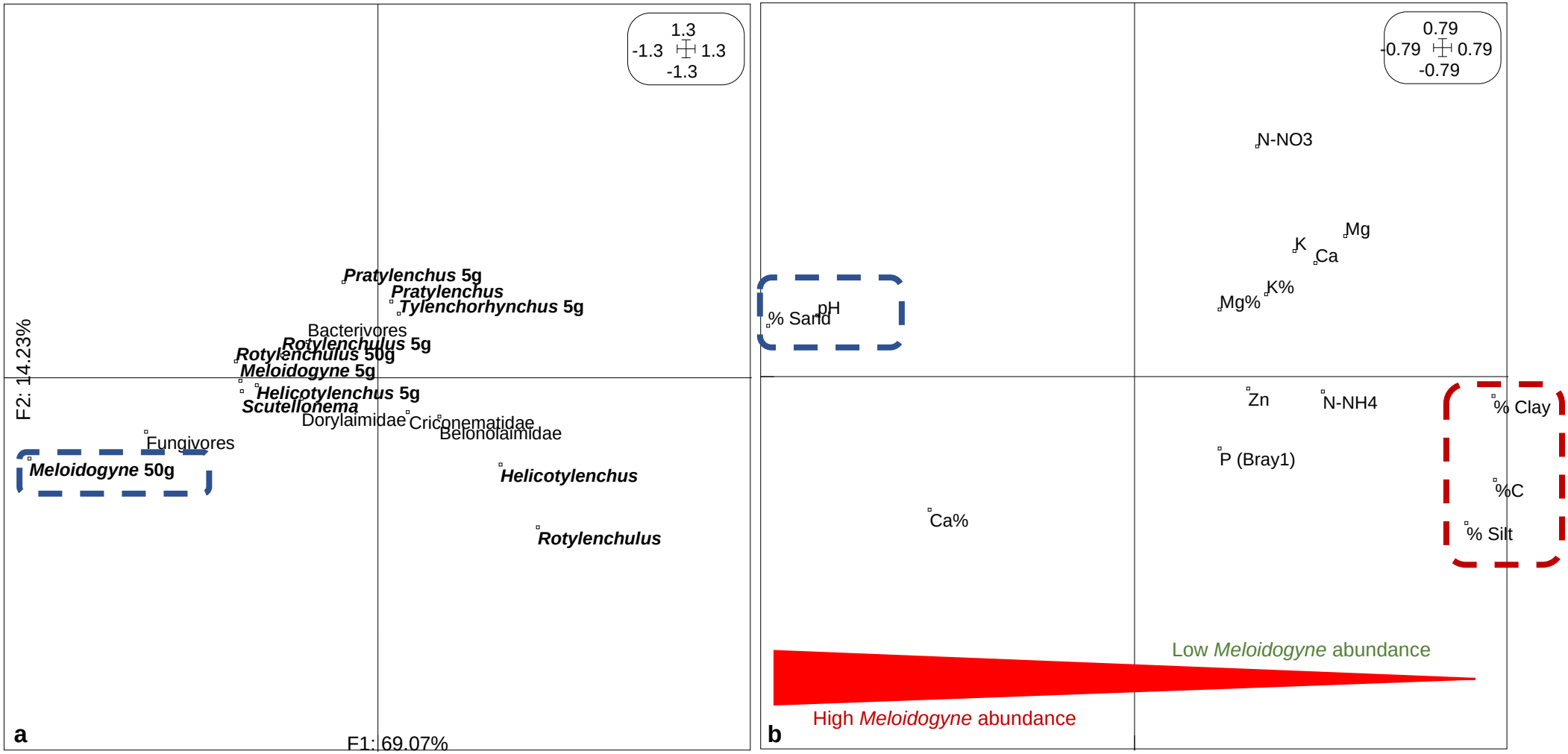
Figs.3.3 a & b) Factorial plane of nematode data (untransformed, real means for plant-parasitic genera and beneficial trophic groups) and **b)** Factorial plane for the 15 localities sampled for nematodes during the 2nd nematode sampling of the 2019 survey in fields of New-Era grain producers. [Localities: 1=Baberspan (North-West), 2=Blesbokfontein (North-West), 3=Bothania (Free-State), 4=Dwaalkraal (North-West), 5= Edenville1 (Free- State), 6=Edenville2 (Free-State), 7=Hoopstad (Free-State), 8=Kaalbass (Gauteng), 9=Klipkop24 (Gauteng), 10=Klipkop530 (Gauteng), 11=Larestryd (North-West), 12=Lyshof (North-West), 13=Putfontein (North-West), 14= Stella (North-West), 15= Virginia (Free-State)].



Figs 3.4 a & b. a) Factorial plane of nematode data (untransformed, real means for plant-parasitic genera and beneficial trophic groups) and **b)** Recalculated co-inertia analysis of soil abiotic parameters associations existing between plant-parasitic genera and beneficial trophic groups and soil abiotic parameters for the 2nd sampling of the 2019 survey as illustrated by first factorial maps.



Figs 3.5 a & b. a) Factorial plane of nematode data (untransformed, real means for plant-parasitic genera and beneficial trophic groups) and **b)** Factorial plan for the 15 localities sampled for nematodes during the 2nd nematode sampling of the 2020 survey in fields of New-Era grain producers. [Localities: 1=Baberspan (North-West), 2=Blesbokfontein (North-West), 3=Bothania (Free-State), 4=Dwaalkraal (North-West), 5= Edenville1 (Free- State), 6=Edenville2 (Free-State), 7=Hoopstad (Free-State), 8=Kaalbass (Gauteng), 9=Klipkop24 (Gauteng), 10=Klipkop 530 (Gauteng), 11=Larestryd (North-West), 12=Lyshof (North-West), 13=Putfontein (North-West), 14 = Stella (North-West),15=Virginia (Free-State)].



Figs 3.6 a & b. a) Factorial plane of nematode data (untransformed, real means for plant-parasitic genera and beneficial trophic groups) and **b)** Recalculated co-inertia analysis of soil abiotic parameters associations existing between plant-parasitic genera and beneficial trophic groups and b soil abiotic parameters for the 2nd sampling of the 2020 survey as illustrated by first factorial maps.

3.4 Discussion

Results of this first nematode survey in maize and sunflower fields of 15 New-Era grain producers provided new insights in terms of the abundance and occurrence of the two predominant PPN genera, *Meloidogyne* and *Rotylenchulus*, thriving in rhizospheres of these crops. Information generated for beneficial nematodes only up to trophic level, also added value to existing information for these two crops. Furthermore, the scale of this survey is comparable to that of the first maize survey done in SA for 14 commercial fields (De Waele & Jordaan, 1988). The New-Era grain producers only rotated maize with sunflower in the 2020 growing season and therefore data was only obtained for the latter season.

It is, furthermore, important to note that only data for 5 g roots (using a sugar-flotation method; Marais *et al*., 2017) were generated for surveys being done prior to the 2000s, while for surveys conducted thereafter data were generated for 5 g and 50 g roots (using the NaOCl method; Riekert, 1995). The latter adapted NaOCl method (Riekert, 1995) was used in the more recent surveys since it has been specifically developed to optimally extract eggs of the sessile endoparasitic nematode genera (e.g., *Meloidogyne* and *Rotylenchulus*) from roots of infected crops. Despite the difference in terms of extraction methods used, the trends with focus on the dominating PPN in roots (whether it is for 5 g or 50 g) of these two crops is discussed below.

Data from the New-Era grain producer survey demonstrated that the sessile, endoparasitic genera most commonly found in roots (5 g & 50 g) of both maize and sunflower were *Meloidogyne* and *Rotylenchulus*, while the migratory endoparasite *Pratylenchus* followed, but in relatively low numbers in 5 g root samples (<5 individuals/5 g roots). *Meloidogyne* and *Rotylenchulus,* dominated due to their superior PV, which were calculated by incorporating abundance and frequency of occurrence (Bekker *et al.,* 2022). Population densities increased for *Meloidogyne* over the sampling period at 73% of the localities and for *Rotylenchulus* at 67% of the localities, which is substantiated by previous reports that showed an increase in abundance of these genera over time (Patel & Patel, 1991; Montasser *et al*., 2013). Interestingly, the highest increases were recorded for two of the localities where sunflower was grown during the last sampling interval of 2020, namely Hoopstad (91% increase for *Meloidogyne* and 69% increase for *Rotylenchulus*) and Dwaalkraal (95% increase for

Meloidogyne and 91% increase for *Rotylenchulus*). Although this data complemented the high susceptibility status of sunflower to *Meloidogyne* (Fourie *et al*., 2017), no record was found in literature confirming the high susceptibility status of sunflower to *Rotylenchulus.*

For the 50 g roots, using the NaOCl extraction method, exceptionally high *Meloidogyne* PV of up to 110344 eggs and J2 and 38463 for sunflower were recorded during the 2nd sampling interval of the 2020 growing season, with these two genera occurring in 100% of the samples. The dominance of *Meloidogyne* spp. in maize roots resulting from this survey agrees with results by Pretorius (2014), who recorded up to 38479 eggs and J2/50 g roots but differed from the low abundance and frequency of occurrence of *Meloidogyne* spp. reported for earlier maize surveys, in Namibia (De Waele *et al*., 1988) and SA (De Waele & Jordaan, 1988a).

For the latter survey only the sugar flotation method was used to extract nematodes from 5 g roots, which may explain the high abundance of *Meloidogyne* spp. recorded from the New-Era grain producer survey using the adapted NaOCl method of Riekert (1995) which was specifically developed to optimally extract eggs of this sessile endoparasitic genus that produces egg masses. Riekert (1996) and Riekert & Henshaw (1998) also demonstrated that high numbers of *Meloidogyne* spp. were isolated from maize roots when these authors used the adapted NaOCl method for the first time in SA to extract *Meloidogyne* eggs and J2 from roots of grain crops in particular. Riekert (1996) reiterated that the abundance and occurrence of *Meloidogyne* spp. in SA have been greatly underestimated and subsequently the damage caused by this genus. Another explanation for the low abundance and occurrence of *Meloidogyne* spp. in maize fields reported from the two SSA countries, Namibia and SA, might be that *Pratylenchus* represented the predominant genus at that time, but that nematode community composition changes occurred with time with *Meloidogyne* becoming predominant during the last two decades.

Although it had lower PV than *Meloidogyne*, *Rotylenchulus* followed in predominance with high values of 6368 and 2129/50 g roots for sunflower and maize, respectively. Nonetheless, the high occurrence and abundance of *Rotylenchulus* in maize roots found in this New-Era grain producer survey is in agreement with a recent report by Bekker *et al*. (2022), who showed that *Rotylenchulus*, following *Meloidogyne* was

especially abundant in fields of commercial producers where conservation agriculture was practiced. However, results from this New-Era grain producer survey are in contrast with the findings of another recent survey done in 78 maize fields (Pretorius, 2016) since during the latter survey *Rotylenchulus* were not recorded for 50 g root samples. Interestingly, during the survey done by Pretorius (2016), *Pratylenchus* followed *Meloidogyne* in predominance in the 5 g root maize samples.

Furthermore, results from this New-Era grain producer survey also disagrees with data from the first maize survey done in commercial maize fields in SA in the 1980s (De Waele & Jordaan, 1988) showing that *Pratylenchus* spp. [*Pratylenchus zeae* Graham, 1951, followed by *Pratylenchus brachyurus* (Godfrey, 1929) Filipjev & Schuurmans-Stekhoven, 1941] were the predominant endoparasites infecting maize crops in 14 commercial fields sampled in SA. For the neighbouring SSA countries Namibia, *Pratylenchus* was also listed as the dominating genus in maize roots, with *P. zeae*, *Pratylenchus penetrans* (Cobb, 1917) Filipjev & Schuurmans-Stekhoven, 1941 and *P. brachyurus* in descending order of dominance (De Waele *et al*., 1988). Listing of *Rotylenchulus* following in predominance after *Meloidogyne* is hence interesting and should be investigated in future.

Concerning the abundance and occurrence of *Rotylenchulus* in sunflower rhizospheres, data from the New-Era grain producer survey showed exceptionally high PV/50 g roots for one of the four fields sampled that was under sunflower cultivation. Unfortunately, only data for 5 g roots were reported for the 1989 survey (Bolton & De Waele, 1989) with *Meloidogyne* being the predominant genus, followed by *Rotylenchulus* which is in agreement with this New-Era grain producer survey. Moreover, of the 15 fields sampled, two of the four sunflower fields had the highest *Rotylenchulus* PV of 6368 and 2129/50 g roots compared to that of the maize fields, with this genus occurring in 56-100% of the fields. Data from earlier maize and sunflower surveys showed that although in low abundance *Rotylenchulus* occurred in nearly all samples (De Waele & Jordaan, 1988; De Waele *et al*., 1988; Bolton *et al*., 1989), which was in contrast with the high abundance of this genus in maize and sunflower fields sampled during this New-Era grain producer survey.

Furthermore, data of the only, dated sunflower survey done in 14 commercial sunflower fields in SA during the late 1980s (Bolton & De Waele, 1989) showed that *Pratylenchus*, followed by *Meloidogyne* were recorded as the predominant PPN genera infecting the roots of the crop. The data generated for this New-Era grain producer survey, in which only four sunflower fields were included, differed from that of the 1989 survey since *Meloidogyne* dominated (high abundance of up to 110344 eggs and J2/50 g roots), and was followed by *Rotylenchulus* and not *Pratylenchus* for which low PV of <5 individuals/5 g roots were recorded. Data from this New-Era grain producer survey hence also do not agree that *Pratylenchus* is abundant in local sunflower production areas as is recorded in literature (Fourie *et al*., 2017). It is, however, important to keep in mind that only a limited number of sunflower fields, four of the 15 fields sampled, were included in this New-Era grain producer survey, and that these sunflower fields were only sampled in the 2020 survey since in the 2019 survey maize were cultivated on all the fields sampled. A survey in sunflower fields of SA should hence be done on a wider scale to confirm such results.

Although *Helicotylenchus* and *Tylenchorhynchus* were also identified from 5 g maize and sunflower roots, their PV were low. Since these two genera represent ectoparasites of which individuals were most probably busy feeding when extractions were done, their presence in roots warrants no further discussion. In terms of PPN soil data, *Rotylenchulus* and *Helicotylenchus* generally dominated in 200 g soil samples, with other PPN genera also being present but in lower abundance and occurrence being *Scutellonema*, *Criconema* and *Belonalaimus* (in order of decreasing PV). The occurrence of the three genera were relatively low agreeing with results of earlier surveys (Keetch & Buckly, 1984; De Waele & Jordaan, 1988; Bolton & De Waele, 1989) and since they are known to commonly occur in low population levels and irregularly in maize fields they are not regarded as constraints to the production of these crops (Louw, 1982; De Waele & Jordaan, 1988a). However, the relatively high PV recorded for *Helicotylenchus* and *Rotylenchulus* during the 1st sampling of the 2019 survey, namely 2270 for *Helicotylenchus* and 1381 for *Rotylenchulus*, is in contrast with low PV for these genera recorded for earlier surveys of maize (De Waele & Jordaan, 1988; De Waele *et al*., 1988) and sunflower (Bolton *et al*., 1989), and should be an indication that their presence in soil samples should be taken cognizance of, and that future studies should aim at elucidating their effects on grain crops.

Soil data for beneficial nematodes showing that bacterivores were predominant followed by fungivores, omnivores and predators are similar to reports for a maize study conducted in SA (Bekker *et al*., 2022) and Kenya (Maina *et al*., 2019). A recent study by Atandi *et al*. (2022) also reported that bacterivores were predominant nematode trophic groups in organic maize and bean fields in SSA. Furthermore, other reports have shown that bacterivores are the most common trophic group in agricultural soils (Djigal *et al*. 2012).

Association data for the 2020 survey yielded interesting information for *Meloidogyne* and *Rotylenchulus*/50 g roots, as well as for bacterivore and fungivores/200 g soil that suggested that these genera and trophic group, respectively, were present in high abundance at locations with high %sand, K (mg/kg), and N-NH_4 (mg/kg) values. These results generally, agreed with those of Bekker *et al*. (2022) who reported a high abundance of *Rotylenchulus* and bacterivore, *Cephalobus* in particular, in maize fields in SA. However, it was in contrast with reports by Bekker *et al*. (2022) who reported high abundance of Hoplolaimidae in maize locations to be associated with elevated % sand, as well as pH and P (mg/kg) values.

Associations for bacterivores and high %sand from the New-Era grain producers also to a certain extent agrees with those of Van Den Hoogen *et al.* (2019) who identified sand content, but also pH as the main drivers in bacterivore community composition. For the 2020 survey high numbers of *Meloidogyne*/50 g roots and fungivores/200 g of soil were present at locations with high %sand and pH values, while the opposite was observed in locations with high %clay, %silt, and %C values. For *Meloidogyne*, these results are consistent with the general trend that population density of this genus increases in sandy soils rather than silt and clay soils (Jaraba-Navas *et al*., 2007). Another study by Curtis *et al*. (2009) also reported that *Meloidogyne* spp. prefer sandy soils for development and reproduction which is in agreement with the data generated for this New-Era grain producers survey.

In conclusion it is important to emphasise that *Rotylenchulus*, following in dominance after *Meloidogyne*, in maize and sunflower rhizospheres as found in this New-Era grain producer survey, and the survey by Bekker *et al.* (2022) warrants further investigation. The role of *Rotylenchulus* in the rhizosphere of grain crops, particularly its impact on

the growth and development, and yield of maize and sunflower, as well as that of other grain crops grown in SA, is unknown (Bekker *et al*., 2022). This study ultimately added novel information about PPN and beneficial nematodes occurring in fields of New-Era grain producers who has to carefully plan to manage the predominant *Meloidogyne* spp. that is known to cause extensive yield losses to maize and other grain crops grown in rotation (Keetch, 1989; Fourie *et al*., 2017; Mc Donald *et al*., 2017).

CHAPTER 4: RESULTS AND DISCUSSION - MORPHOLOGICAL AND MOLECULAR IDENTIFICATION

4.1 Results

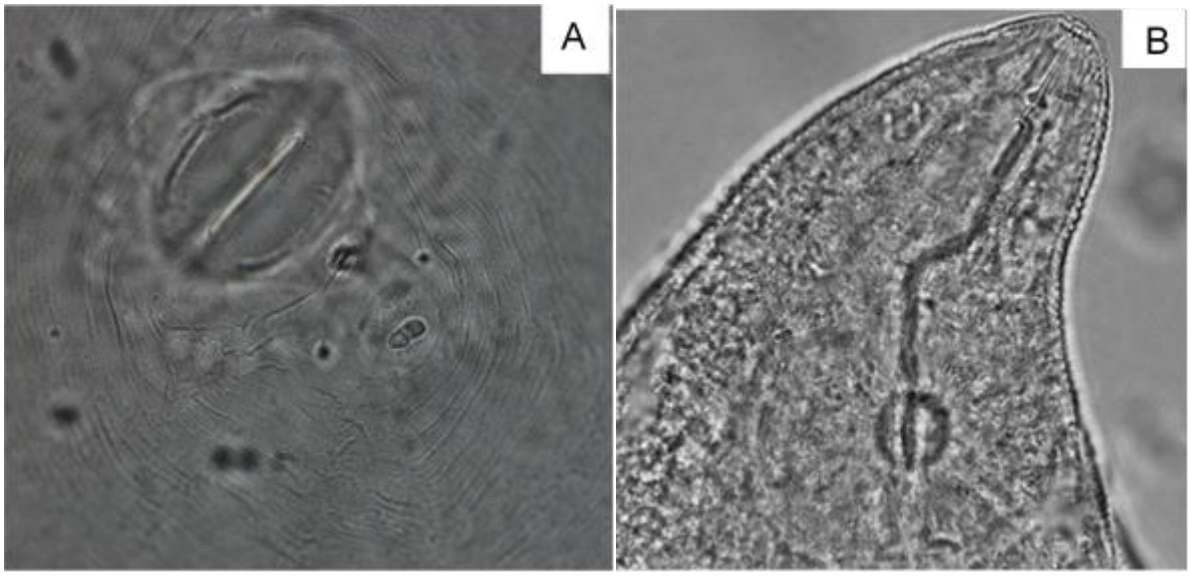
Species of *Meloidogyne* have distinct morphological characteristics that allow them to be distinguished from one another (Table 2.2), while state-of-the-art biochemical and molecular techniques are available to complement morphological identifications enabling accurate characterization of species. Using both morphological and molecular techniques, results from this study are given below.

4.2.1 Morphological/classical identification

Using the general characteristics for perineal patterns and oesophagi of mature females, three *Meloidogyne* spp. (Table 2.2), they were identified as *Meloidogyne enterolobii* Yang & Eisenback, 1983, *Meloidogyne incognita* (Kofoid and White, 1919) Chitwood, 1949, and *Meloidogyne javanica* (Treub, 1885) Chitwood, 1949 (Table 4.2). No *Meloidogyne arenaria* (Neal, 1889) Chitwood, 1949, females were identified from the 15 localities using the morphological approach.

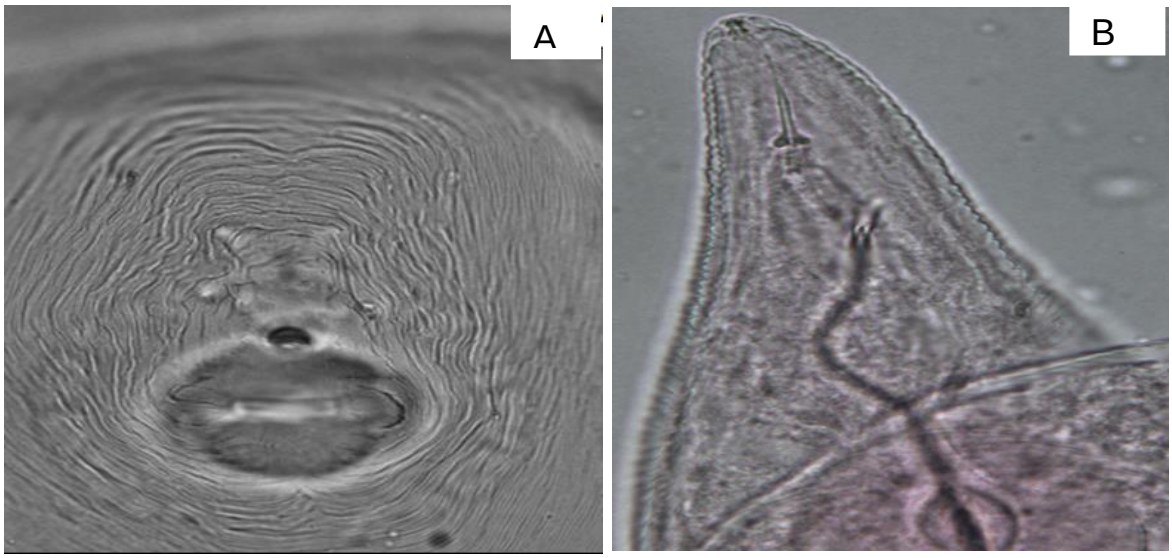
Meloidogyne incognita dominated in terms of occurrence in single-species populations at seven of the localities (1, 2, 3, 4, 5, 10 & 12), followed by *M. javanica* occurring as a single-species population at one locality (13) (Table 4.2). Mixed communities of *M. incognita* and *M. javanica* occurred at six localities (6, 7, 8, 9, 11 & 14), while a mixed communities of all three species (*M. incognita, M. javanica & M. enterolobii* occurred at one locality (15).

Mature female individuals of *M. enterolobii* studied had round to oval shaped perineal patterns with a moderately high to high dorsal arch, and an apex that was rounded but square in some specimens (Fig. 4.1A). Lateral lines were absent or not distinct, while distinct phasmids with striae was visible at the tail-end. Furthermore, striae were absent in the peri-vulval area, while those on the ventral part of the pattern were generally finer and smoother (Table 2.2). Since no information is available regarding the form and shape of the oesophagi of females, the lumen of the oesophagus as well as the pro- and metacorpus (Fig. 4.1B), could not be used to identify this species.



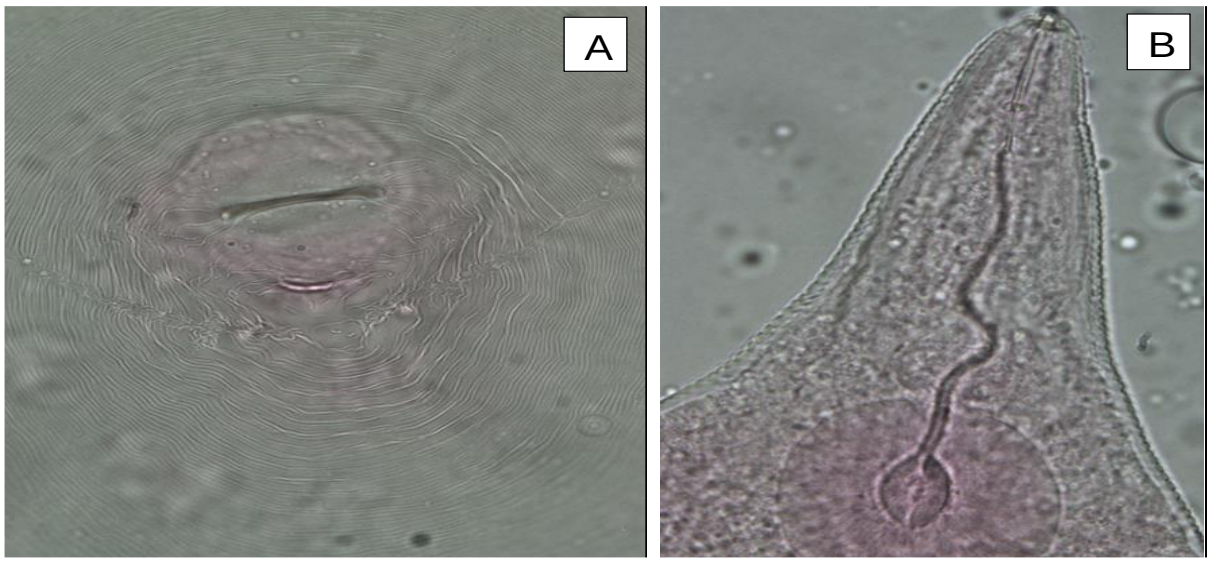
Figs 4.1 A perennial pattern that resembles **(A)** a *Meloidogyne enterolobii* female specimen (100x magnification using a compound microscope and the oesophageal region **(B)** of the same specimen (100x magnification using a compound microscope) showing the stylet and lumen of the oesophagus (Photos: Lindokuhle Qwabe, North-West University).

Females of *M. incognita* studied had a circular to oval shaped perineal pattern, a medium high to high dorsal arch and a rounded to broadly squarish apex (Fig. 4.2A). Disordered phasmids were visible in the tail terminus, but not prominent as was the case for *M. enterolobii* females. The pattern of striae was visible as a zigzag, wavy, and/or smooth lines. The lumen lining that expanded and then narrowed towards spheroid was visible in some specimens, with the metacorpus generally present as an oval shape (Fig. 4.2B). The general oesophageal identification (Fig.3.4B) was made upon the presence of a spheroid metacorpus lining.



Figs. 4.2 A perennial pattern that resembles **(A)** a *Meloidogyne incognita* female specimen (100x magnification using a compound microscope) and the oesophageal region **(B)** of the same specimen (100x magnification using a compound microscope) showing the stylet and lumen of the oesophagus (Photos: Lindokuhle Qwabe, North-West University).

Females of *M. javanica* had a circular to oval shaped perineal pattern, a low to medium-high dorsal arch and a squarish to broadly rounded apex (Fig. 4.3A). Rectal punctuations were also visible, as well as distinct double lateral lines; areas above these lines were not bulged out (Yang and Eisenback, 1983; Eisenback 1985; Kleynhans, 1991; Brito *et al*., 2004; Karssen *et al*., 2013; Agenbag, 2016; Rashidifard *et al*., 2019c). A spherical metacorpus lining was present in most individuals, with the lumens of their oesophagi generally being cylindrical but also expanding and/or narrowing in front of ovoid (Fig. 4.3B).



Figs. 4.3 A perennial-pattern that resembles **(A)** a *Meloidogyne javanica* female specimen (100x magnification using a compound microscope) and the oesophageal region **(B)** of the same specimen (100x magnification using a compound microscope) showing the stylet and lumen of the oesophagus (Photos: Lindokuhle Qwabe and Driekie Fourie, North-West University).

4.2.2 Molecular identification

Using the SCAR-PCR method by applying species-specific primers for *M. arenaria* (420 bp fragment amplified), *M. enterolobii* (420 bp fragment amplified), *M. incognita* (399 bp fragment amplified) and *M. javanica* (670 bp fragment amplified), respectively, resulted in identification of these four species (Figs. 4A-D; Table 2.2).

Meloidogyne incognita dominated in terms of occurrence since it was present at seven localities showing amplification of the 399 bp fragment which agreed with that of the species-specific primer (Santos *et al*., 2017) and the standard used (Fig. 4A-D; Table 4.2). The species occurred as either single-species populations (at localities 6, 8 & 10) or mixed communities with: *M*. *javanica* at localities 3 & 11; *M. arenaria* at localities 3, 7 & 14; *M. enterolobii* and *M. javanica* at locality 15. Followed by *M. incognita* found as pure populations at localities 2 & 4 (Figs. 4A-D; Table 2.2).

Meloidogyne javanica was second in dominance occurring at six of the localities as shown by amplification of the 670 bp fragment which agreed with that of the species-specific marker (Zijlstra *et al*., 2000) and the standard used (Figs. 4A-D; Table 2.2). This species occurred as single-species populations at localities 6, 8 & 10 and in mixed

communities with: *M. arenaria* at locality 9; and *M. incognita* at localities 3 & 11. *Meloidogyne arenaria*, third in dominance, was present at four of the localities as indicated by amplification of DNA representing the 420 bp fragment that was like that of the species-specific marker (Zijlstra *et al*., 2000) and the standard used (Figs. 4A-D; Table 2.2). This species occurred only in mixed communities with: *M. incognita* at localities 7 & 14; *M. javanica* at locality 9; and *M. enterolobii* and *M. incognita* at locality 15.

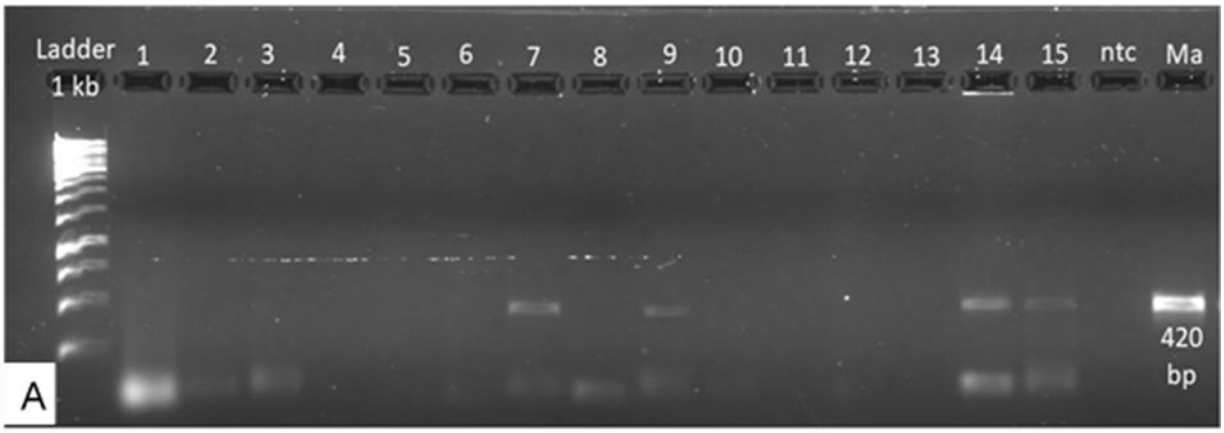
Meloidogyne enterolobii was the least dominant species occurring at two localities with the 200 bp fragment being amplified which agreed with that of the species-specific marker (Long *et al*., 2006) and the standard used (Figs. 4A-D; Table 2.2). This species occurred as a single species at locality 12 and in mixed communities with *M. arenaria* and *M. incognita* at locality 15.

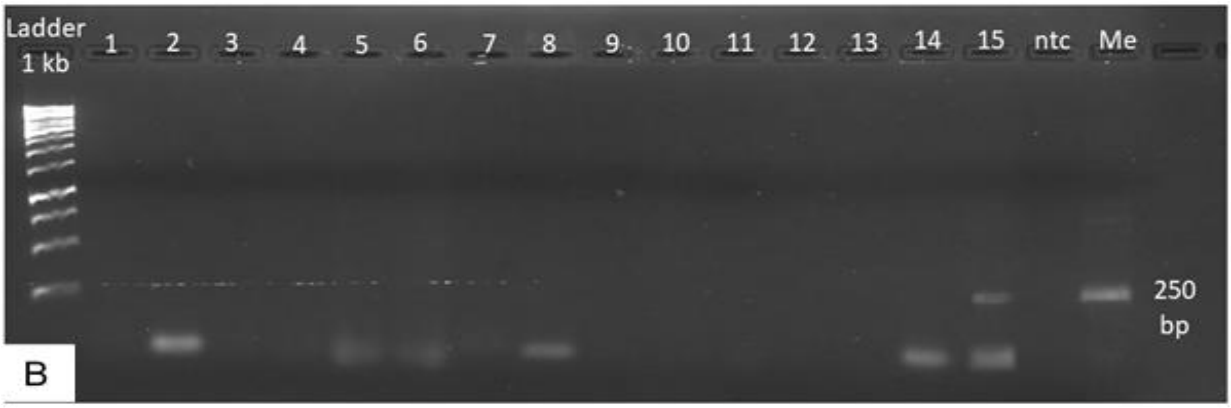
Since localities 1, 5, 12, & 13 did not show any amplification of *Meloidogyne* spp. DNA during the first analysis, mature females were again isolated from infected tomato roots in which the species were reared *in vivo* (see section 2.4.1), and the SCAR-PCR analysis repeated. This second analysis showed no DNA bands being amplified for *M. arenaria* (420 bp fragments), *M. incognita* (399 bp fragments) (Fig. 4.4E; Table 2.2) and *M. javanica* (670 bp fragments) for localities 1, 5 & 13 (Fig. 4.4G; Table 2.2). However, amplification of DNA of *M. enterolobii* females was visible for locality 12 with the 200 bp fragment being amplified and corresponding with that of the species-specific primer (Long *et al*., 2006) and the standard used (Fig. 4.4F; Table 2.2).

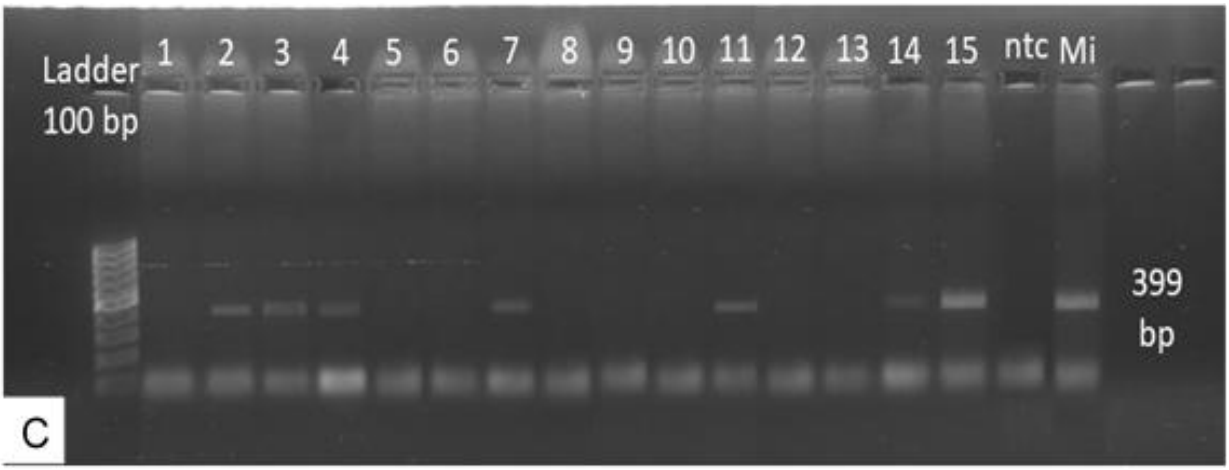
Table 4.2 Results obtained using morphological and molecular techniques to identify *Meloidogyne* spp. present at 15 localities sampled of grain producers in the Free State, Gauteng, and North-West provinces of South Africa during the 2018/19 and 2019/20 growing seasons. The percentage shows the contribution by each of the *Meloidogyne* spp. species to the population and/or community identified morphologically from each of the 15 localities.

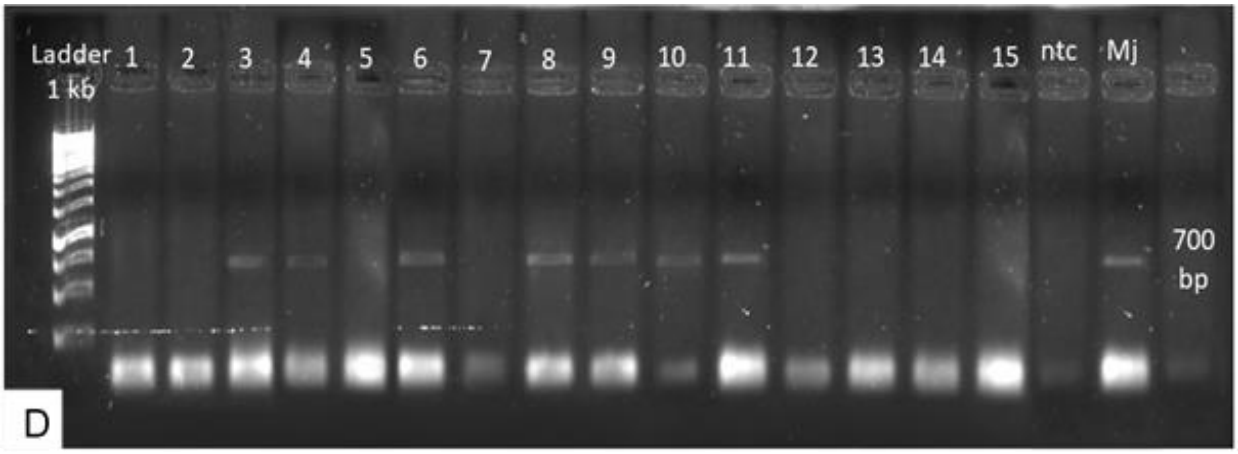
Locality & crop	Species identified using morphological data	*Meloidogyne* spp. identified using sequence characterised amplified regions-polymerase chain reaction (SCAR-PCR)
1 (M/SF)	*M. incognita (100%)*	No DNA amplified
2 (M/M)	*M. incognita (100%)*	*M. incognita*
3 (M/M)	*M. incognita (100%)*	*M. incognita* & *M. javanica*
4 (M/SF)	*M. incognita (100%)*	*M. incognita*
5 (M/M)	*M. incognita (100%)*	No DNA amplified
6 (M/M)	*M. incognita (50%)* *M. javanica (50%)*	*M. javanica*
7 (M/SF)	*M. incognita (60%)* *M. javanica (40%)*	*M. incognita* & *M. arenaria*
8 (M/M)	*M. incognita (83%)* *M. javanica (17%)*	*M. javanica*
9 (M/M)	*M. incognita (83%)* *M. javanica (17%)*	*M.arenaria* & *M. javanica*
10 (M/M)	*M. incognita (100%)*	*M. javanica*
11 (M/M)	*M. incognita (67%)* *M. javanica (33%)*	*M. incognita* & *M. javanica*
12 (M/M)	*M. incognita (83%)*	*M. enterolobii*
13 (M/M)	*M. javanica (100%)*	No DNA amplified
14 (M/M)	*M. incognita (86%)* *M. javanica (14%)*	*M. incognita* & *M. arenaria*
15 (M/SF)	*M. enterolobii (57%)* *M. incognita (43%)*	*M. incognita, M. arenaria* & *M. enterolobii*

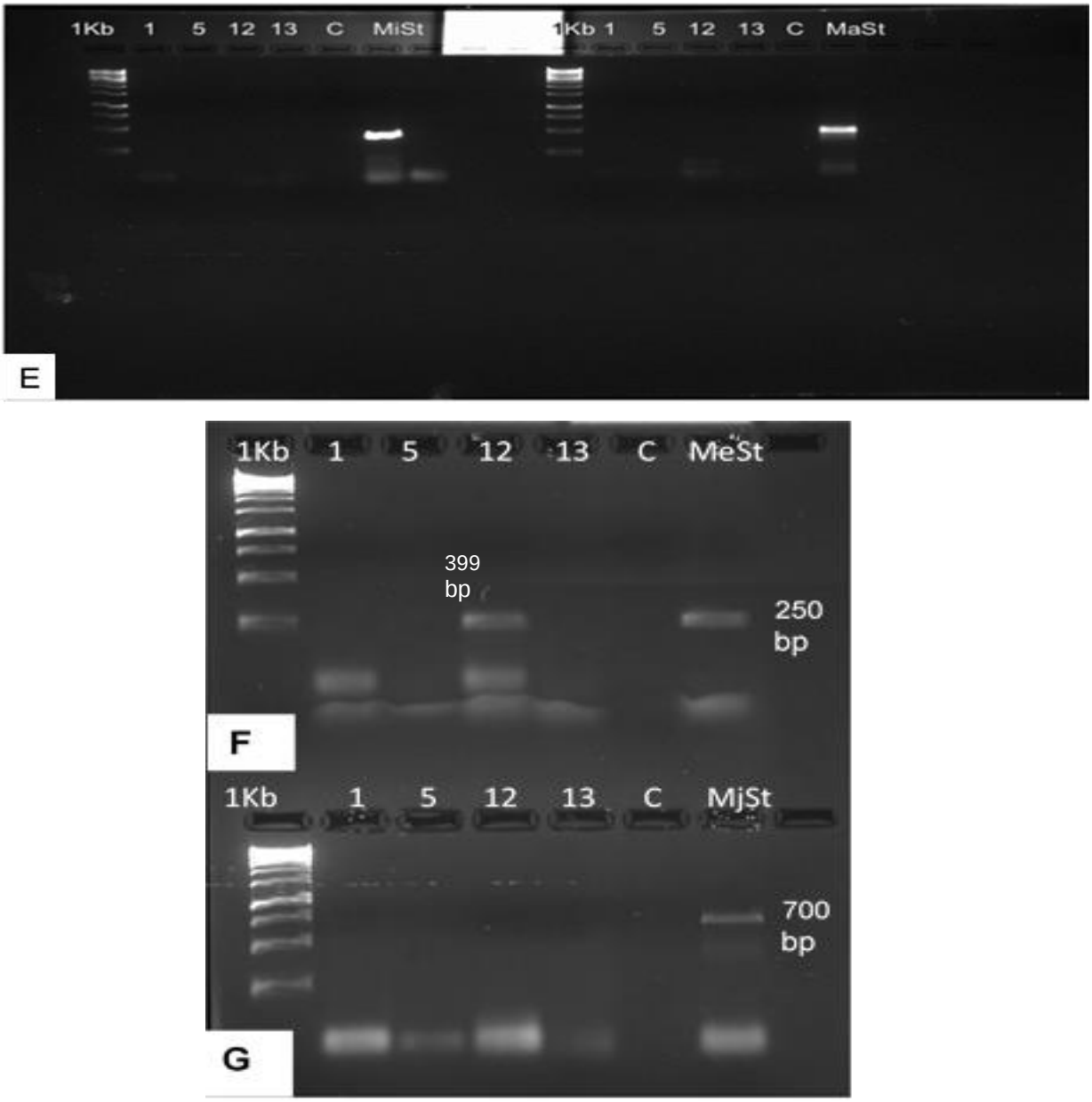
1=Baberspan (North-West), 2=Bleksbofotein (North-West), 3=Bothania (Free-State), 4=Dwaalkraal (North-West), 5= Edenville1 (Free- State), 6=Edenville2 (Free-State), 7=Hoopstad (Free-State), 9=Kaalbass (Gauteng), 9=Klipkop24 (Gauteng), 10=Klipkop530 (Gauteng), 11=Larestryd (North-West), 12=Lyshof (North-West), 13=Putfontein (North-West), 14=Stella (North-West) 15=Virginia (Free-State). *M/M = Maize grown in 2019 and 2020; M/SF = Maize grown in 2019 and sunflower in 2020.


Ladder
1 kb
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 ntc Ma
420
bp
A


Ladder
1 kb
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 ntc Me
250
bp
B


Ladder
100 bp
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 ntc Mi
399
bp
C


Ladder
1 kb
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 ntc Mj
700
bp
D



Figs. 4.4 Gel photographs of DNA-fragment amplification products of *Meloidogyne* females obtained from roots of grain crops using the Sequence Characterised Amplified Region-Polymerase Chain Reaction (SCAR-PCR). **(A)** *M. arenaria*; **(B)** *M. enterolobii*; **(C)** *M. incognita* and **(D)** *M. javanica*. A 1kb DNA ladder was used for Ma, Me, and Mj and a 100 pb ladder was used for Mi denotes the DNA of females from the standard populations *M. arenaria*, *M. enterolobii*, *M. incognita* and *M. javanica*, respectively, while ntc indicates the non-template control. **(E)** *M. arenaria* and *M. incognita*, **(F)** *M. enterolobii* and **(G)** *M. javanica* A 1kb DNA ladder was used for MaSt, Me and MiSt DNA of females from the standard populations *M. arenaria*, *M. enterolobii* and *M. incognita* respectively, while C indicates the non-template control.1=Baberspan (North-West), 2=Bleksbofotein (North-West), 3=Bothania (Free-State), 4=Dwaalkraal (North-West), 5= Edenville1 (Free-State), 6=Edenville2 (Free-State),

7=Hoopstad (Free-State), 9=Kaalbass (Gauteng), 9=Klipkop24 (Gauteng), 10=Klipkop530 (Gauteng), 11=Larestryd (North-West), 12=Lyshof (North-West), 13=Putfontein (North-West), 14=Stella (North-West),15=Virginia (Free-State).

4.3 Discussion

The presence of four *Meloidogyne* spp. *viz*. *M. arenaria, M. enterolobii, M. incognita* and *M. javanica* were identified from the 15 localities sampled during this New-Era grain producer survey using female morphological features (mainly perineal-patterns, but also oesophageal features to a lesser extent) and the molecular SCAR-PCR technique. The three *Meloidogyne* spp. reported to infect and damage local maize and other grain crops grown in rotation, namely *M. arenaria*, *M. incognita, M. javanica* (Mc Donald *et al*., 2017; SAPPNS) as well as *M. enterolobii* has been identified from the 15 fields of New-Era grain producers sampled. This second report of *M. enterolobii* being found in maize fields in SA, complementing the first report of Pretorius (2016) who identified it from a maize field in the Mpumalanga province is hence an addition to the South African Plant Parasitic Nematode database (SAPPNS) for the localities Putfontein and Stella (North-West province).

Applying both the classical approach and the SCAR-PCR analysis, single-species populations as well as mixed communities of more than one species could be identified. The two methods complement each other, but also showing discrepancies between results obtained using these approaches and were also reported by Visagie *et al*. (2018) and Matlala (2020).

While only three species were identified with the classical approach, namely *M. incognita, M. javanica* and *M. enterolobii*, the SCAR-PCR enabled identification of a fourth species, namely *M. arenaria*. This represents a useful example why more than one identification method should be used since morphological identification is challenging, even for trained and experienced experts (Adam *et al*., 2007; Brito *et al*., 2004; Carneiro *et al*., 2017). With the aid of the SCAR-PCR assay, one more species (*M. arenaria*) were identified compared to using the classical approach. It is important to keep in mind that two separate sets of females have been randomly picked for the morphological *vs*. the molecular analysis, which is suggested to have contributed to the fact that the set of females picked for one analysis did not necessarily resemble

the species present in the set of females selected for the other analysis. It is further agreed with Adam *et al*. (2007), Brito *et al*. (2004) and Carneiro *et al*. (2017) that distinguishing among *Meloidogyne* spp., particularly those in the *Mi*-group, is difficult due to these species having similar and/or overlapping perineal-pattern characteristics. Identifying particularly *M. enterolobii* using the classical approach posed another challenge, but it is agreed with Visagie *et al*. (2018) and Rashidifard *et al*. (2019), that the presence of pronounced, clearly visible phasmids near the rectum of *M. enterolobii* females studied, was a valuable guideline opposed to phasmids of *M. incognita* which could be seen but were not pronounced and disordered. It is hence agreed with Yang and Eisenback (1985) and Rashidifard *et al*. (2019a) that this is a valuable characteristic to distinguish between the two species.

The dominance of *M. incognita*, followed by *M. javanica* in the maize production area studied agrees with previous reports for South Africa (Riekert, 1996; Riekert & Henshaw, 1998; Mc Donald *et al*., 2017; Pretorius, 2018). The presence of these two species in local grain production areas represent the two most commonly occurring species infecting grain crops such as maize, sunflower (Fourie *et al*., 2017) and other grain crops either as single or mixed communities (Kleynhans, 1991; Fourie *et al*., 2017; Mc Donald *et al*., 2017; Visagie *et al*., 2018). Karssen (2013) also reported the common occurrence of more than one *Meloidogyne* spp. from agricultural fields on a global scale. Furthermore, recently, Pretorius (2016) also found *M. incognita*, followed by *M. javanica* (in order of dominance) occurring in mixed communities in fields where maize and sunflower were rotated, agreeing with results of this New-Era grain producer survey. Important to note is that various grain crops are susceptible hosts for these two species which will lead to optimal reproduction and increased population densities in grain fields. Therefore, it is crucial that the host status of genotypes of grain crops, as well as other crops grown in rotation with maize, be assessed so that producers can use poor host or resistant genotypes to mitigate damage caused by these two commonly occurring species.

The presence of *M. arenaria* and *M. enterolobii* occurring to a lesser extent at the 15 localities sampled also agrees with reports by Mc Donald *et al*. (2017) and Pretorius (2016), respectively. The presence of these two species in New-Era grain producers' fields should also be taken cognisance of with future research activities proposed to also focus on their pathogenicity. For example, the screening of crop genotypes for

their host status towards these respective species should also be addressed since they have wide host ranges (Onkendi & Moleleki, 2013; EPPO, 2014; Visagie *et al*., 2018; Rashidifard *et al*., 2019a & b), which is similar to those of *M. incognita* and *M. javanica*. However, *M. enterolobii* is known to be more aggressive and pathogenic than its thermophilic counterpart species (Brito *et al*., 2004) especially since it deactivates resistance that is effective in crops against other species (mainly *M. arenaria*, *M. incognita* and *M. javanica*) (Kiewnick *et al*., 2009). Therefore, priority should be given to the generation of knowledge to elucidate the occurrence of *M. enterolobii* in local grain crop production areas. Such an approach will assist producers to be knowledgeable about the species, plan properly and apply timely and effective management strategies to prevent extensive yield losses.

The occurrence of these four *Meloidogyne* species in mostly mixed communities at 50% of the localities sampled, is not surprising since it is not uncommon for two or more *Meloidogyne* spp. to co-exist in mixed populations (Kleynhans, 1991). *Meloidogyne incognita* and *M. javanica* has for example generally been found to occur in mixed populations in crop fields in SA (Kleynhans *et al*., 1996). Furthermore, the two *Meloidogyne* spp. are often present in mixed populations in local maize fields (Riekert, 1996; Riekert & Henshaw, 1988; Mc Donald *et al*., 2017), while *M. arenaria* is known to occur too, but to a lesser extent than the two former species (Agenbag, 2016). *Meloidogyne enterolobii* has also been found to occur in a mixed community with *M. incognita* in a maize field in the Highveld region of Mpumalanga (Pretorius, 2016), while Visagie *et al*. (2018) and Rashidifard *et al*. (2019a & b) also identified it in mixed communities with other commonly occurring *Meloidogyne* spp. in roots of various crops sampled in SA.

The lack of amplified DNA from females isolated from three of the localities (1, 5 & 13) could be that specific-species primers for thermophilic species other than those of the four species (namely *M. arenaria*, *M. enterolobii*, *M. incognita* and *M. javanica*) used in this study should be included, and also those for cryophilic species (*Meloidogyne chitwoodi* Golden, O'Bannon, Santo & Finley, 1980, *Meloidogyne fallax* Karssen, 1996 and *Meloidogyne hapla* Chitwood, 1949). The inability of identifying *Meloidogyne* spp. from these localities using the SCAR-PCR method accentuates that the single species populations of *M. incognita* (for localities 1 & 5) and *M. javanica* (locality 13) identified using the classical approach may in fact consist of mixed communities with other

species present too, than only those identified. Other techniques could also be used to elucidate the identity of the *Meloidogyne* spp. occurring at these three localities, for example the isozyme approach as reported by dos Santos *et al*. (2019) that lead to identification of species that could not be identified using the SCAR-PCR method in their study.

Application of DNA sequencing using various genes such as cytochrome oxidase sub-unit I (COI), cytochrome oxidase sub-unit II (COII), NADH dehydrogenase 5' (NADH5), genotyping by sequencing (GBS) and others, as demonstrated by Rashidifard *et al*. (2018 & 2019b) in particular is a valuable approach to follow to elucidate the presence of the species that could not be identified in this current study using the SCAR-PCR.

Results generated from New-Era grain producers' production areas revealed information that is novel and crucial for proper planning of grain crop production in fields where mostly mixed communities of *Meloidogyne* spp. occur. While confirming the predominance of *M. incognita* and *M. javanica* in the maize and sunflower fields, the presence of both *M. arenaria* and *M. enterolobii* has been expanded as a result of this study in the New-Era grain producer's fields. The presence of mixed communities of *Meloidogyne* spp. representing two and/or three species as was found in this study, accentuates that nematologists will find it challenging to recommend dedicated and effective management strategies to mitigate their damage to grain crops. Furthermore, more than one identification approach should be utilised to accurately identify *Meloidogyne* spp., which was proven with this study since *Meloidogyne* spp. could not be identified at three of the localities with the two methods used. This calls for a third method or even more methods to be applied to elucidate the identity of such unidentified species.

CHAPTER 5: CONCLUSION

5.1 GENERAL CONCLUSION

This survey, initiated in response to requests from New-Era grain producers by GrainSA, aimed to generate empirically based information on the abundance and diversity of plant-parasitic nematode (PPN) assemblages in fields of New-Era grain producers. The first of two objectives were to determine the abundance, diversity, and occurrence of PPN species in fields of New-Era grain producers situated in three provinces of South Africa (Free-State, North-West and Gauteng) by means of a baseline study, with focus on *Meloidogyne* spp. In addition, the existence of associations between nematodes and selected abiotic parameters have been investigated. The second objective constituted that the identity of *Meloidogyne* spp. that were present in the fields sampled, were studied using morphological and molecular methods.

5.2 TESTING OF THE HYPOTHESES

The following hypotheses were tested and are discussed:
i) The predominant PPN genus in fields of the New-Era grain producers was foreseen to be root-knot nematodes (RKN), while it was also expected that associations between nematodes (PPN and beneficial) and some of the selected abiotic parameters will be evidenced.

Results from this New-Era grain producer survey, done over four sampling intervals and two consecutive growing seasons, showed that *Meloidogyne* Göldi, 1892 (root-knot nematodes) was the predominant genus present in the roots of maize (*Zea mays* L.) and sunflower (*Helianthus annuus* L.) sampled. The interesting part is that controversy was evidenced for the PPN genus ranked as the 2nd in terms of dominance in both maize and sunflower fields of the New-Era grain producer survey. Listing of *Rotylenchulus* Linford & Oliveira, 1940, in 2nd place after *Meloidogyne*, contradicted results by Pretorius (2016) and Mc Donald *et al*. (2017) whom both listed *Pratylenchus* Filipjev, 1936, following *Meloidogyne* in dominance in maize fields in South Africa. Nonetheless, the 2nd ranked *Rotylenchulus* agreeing with findings from a recent maize survey (Bekker *et al*., 2022) who also reported this genus, following *Meloidogyne*, in

maize fields under conservation agriculture. Similarly for sunflower, *Rotylenchus* also ranked 2nd in terms of dominance a result of the New-Era grain survey representing a first report for the crop. Use of the adapted NaOCl method that is specifically developed to extract eggs of sessile, endoparasitic genera such as *Meloidogyne* and *Rotylenchulus* is suggested to have contributed to increased knowledge and expertise to distinguish the eggs of these two genera successfully as was demonstrated by Bekker (2016).

The associations worth mentioning as a result of this New-Era producer survey are those existing for high *Meloidogyne* and *Rotylenchulus* abundance as well as high fungivore abundance occurring at localities with soils containing high %sand. This phenomenon has been reported by Jaraba-Navas *et al*. (2007) and Curtis *et al*. (2009) accentuating those problems caused by the two mentioned PPN genera is expected to be higher in sandy soils. It should serve as a warning to producers to take cognisance of this phenomenon and implement timely and effective management strategies in such fields. More research is, however, needed to elucidate the affinity of fungivores for sandy soils.

Since *Meloidogyne* was found to be the predominant PPN genus infecting maize and sunflower in fields of the New-Era grain producers sampled, the first part of the hypothesis is accepted. Also, since associations were found among nematodes and selected abiotic parameters, the second part of the hypothesis can also be accepted.

ii) The most commonly occurring and predominant root-knot nematode species expected to be identified from the 15 fields of farmers were *Meloidogyne incognita* (Kofoid and White, 1919) Chitwood, 1949, and *Meloidogyne javanica* (Treub, 1885) Chitwood, 1949.

Morphological and molecular identification of *M. incognita* as the most common species, followed by *M. javanica* and *Meloidogyne arenaria* (Neal, 1889) Chitwood, 1949, and *Meloidogyne enterolobii* Yang & Eisenback, 1983, to a lesser degree, is consistent with existing literature at least for the first three species (Bekker, 2016; Mc Donald *et al*., 2017). Interestingly, Pretorius (2016) first reported *M. enterolobii* infecting maize in South Africa, with the notification from this New-Era grain producer survey being the second for this species for local maize. The dominance of

communities that contained more than one *Meloidogyne* spp., opposed to single-species populations, from this New-Era grain producer survey is also in agreement with previous reports for maize and other grain crops grown in South Africa (Pretorius, 2016; Mc Donald *et al*., 2017; Visagie *et al*., 2018; Rashidifard *et al*., 2019). The presence of more than one species in a maize or sunflower field is, however, an indication that producers might find it a challenge to effectively manage *Meloidogyne* in their fields.

The predominant species found infecting maize and sunflower crops in the fields sampled of the New-Era grain producers in this study, namely *M. incognita* and *M. javanica*, agree with the stated hypothesis and therefore it is accepted.

5.3 FUTURE RECOMMENDATIONS

i) The present study revealed new information about the occurrence of *Rotylenchulus* in local sunflower fields, while its presence in maize fields in high numbers is only reported for the 2nd time, after Bekker *et al.* (2022), for this New-Era grain producer survey. Therefore, it is agreed with the latter authors that more extensive studies are required to investigate the impact of *Rotylenchulus* on grain crops, e.g., its impact on the growth and development, and yield of maize and sunflower 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 PPN as well as listing of new species since this will impact substantially on production of such crops and food security.

iii) The highly efficient molecular technique, SCAR-PCR, could not detect *Meloidogyne* spp. from three of the 15 localities included in this study. Therefore, it could be helpful to use various classical and molecular identification methods when identifying *Meloidogyne* spp., and those of other genera, in order to ensure precise and correct species identification. This will contribute towards more dedicated management strategies being employed to mitigate the damage by the economically important PPN.

6. REFERENCES

Abafe, E.A. 2021. Market participation of smallholder sunflower farmers in North-West province, South Africa. University of South Africa, Mahikeng, South Africa. {PhD Thesis}.

Abd-Elgawad, M.M. & Askary, T.H. 2020. Factors affecting success of biological agents used in controlling the plant-parasitic nematodes. *Egyptian Journal of Biological Pest Control*, 30:1-11. https://doi.org/10.1186/s41938-020-00215-2.

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*, 56:190-197.

Agenbag, M. 2016. Identification and pathogenicity of South African *Meloidogyne* species. North-West University, Potchefstroom, South Africa. {MSc Thesis}.

AGOGTR. 2008. The biology of *Zea mays* L. spp. mays (maize or corn). Australian Government Office of the Gene Technology Regulator. Available at http://www.ogtr.gov.au/internet/ogtr/publishing.nsf/content/maize3/$FILE/biologymaize08_2.pdfxhcc (Accessed 5 April 2021).

Agri-Intel. 2022. Agrochemical information. Available at http://www.agri-intel.com (Accessed 4 October 2022).

Alby, T., Ferris, J. M. & Ferris, V. R. 1983. Dispersion and distribution of *Pratylenchus scribneri* and *Hoplolaimus galeatusin* soybean fields. *Journal of Nematology*, 15:418-426.

Amarantha, B. S. & Krishnappa, K. 1990. Plant parasitic nematodes associated with sunflower in Karnataka. *Indian Journal of Nematology*, 20: 193-196.

Atandi, J.G., Adamtey, N., Kiriga, A.W., Karanja, E.N., Musyoka, M.W., Matheri, F.M., Tanga, C.M., Coyne, D.L., Fiaboe, K.K., Bautze, D. & Haukeland, S. 2022. Organic maize and bean farming enhances free-living nematode dynamics in sub-Saharan Africa. *Agriculture, Ecosystems and Environment*, 327: 107846.

Askary, T.H., Waliullah, M.I.S. & Gupta, S. 2012. Population fluctuation of plant-parasitic nematodes associated with pome, stone and nut fruit nurseries. *Annals of Plant Protection Sciences* 20: 265-267.

Assoumana, B.T., Habash, S., Ndiaye, M., Puije, G. van der, Sarr, E., Adamou, H., Grundler, F. M. W. & Elashry, A. 2017. First report of the root-knot nematode *Meloidogyne enterolobii* parasitising sweet pepper (*Capsicum annuum*) in Niger. *New Disease Reports*, 36:18. http://dx.doi.org/10.5197/j.2044- 0588.2017.036.018.

Aung, T., Windham, G.L. & Williams, W.P. 1990. Reproduction of *Meloidogyne incognita* on open-pollinated maize varieties. *Journal of Nematology*, 22:651-653.

Awata, L.A., Tongoona, P., Danquah, E., Ifie, B.E., Mahabaleswara, S.L., Jumbo, M.B., Marchelo-D'ragga, P.W. & Sitonik, C. 2019. Understanding tropical maize (*Zea mays* L.): the major monocot in modernization and sustainability of agriculture in sub-Saharan Africa. *International Journal of Advance Agricultural Research*, 7: 33-77. https://doi.org/10.33500/ijaar.2019.07.004.

Back, M.A., Haydock, P.P.J. & Jenkinson, P. 2002. Disease complexes involvingplant-parasitic nematodes and soil-borne pathogens. *Plant Pathology*, 51:683-697.

Bekker, S. 2016. Nematode communities: Bio-indicators of soil quality in conventional and conservation agricultural cropping systems. North-West University, Potchefstroom, South Africa. {PhD Dissertation}.

Bekker, S., Fourie, H., Rashidifard, M., Daneel, M.S., Shokoohi, E. & Nel, A. 2016. Discriminating between the eggs of two egg-mass producing nematode genera using morphometric and molecular techniques. *Journal of Nematology,* 18: 1119-1123.

Berry, S.D., Fargette, M., Spaull, V.W *et al*., 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 Cellular Probes*, 22:168-176.

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. https://doi.org/https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2619761/.

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. CAB International Publishing. pp. 98-118.

Blok, V.C., Wishart, J., Fargette, M., Berthier, K. & Phillips, M. 2002. Mitochondrial DNA differences distinguishing *Meloidogyne mayaguensis* from the major species of tropical root-knot nematodes. *Nematology*, 4:773-781.

Boema, H.R. & Husey, R.S.1992. Breeding plants for resistance to nematodes. *Journal of Nematology*, 24:242-252.

Braun-Kiewnick, A. & Kiewnick, S. 2018. Real-time PCR, a great tool for fast identification, sensitive detection and quantification of important plant-parasitic nematodes. *European Journal of Plant Pathology*, 152:271-283. https://doi.org/10.1007/s10658-018-1487-7.

Bogale, M., Baniya, A. & DiGennaro, P. 2020. Nematode identification techniques and recent advances. *Plants Journal*, 9:1260. https://doi.org/10.3390/plants9101260.

Bolton, C.H.E.R.Y.L. & De Waele, D. 1989. Host suitability of commercial sunflower hybrids to *Pratylenchus zeae*. *Journal of Nematology*, 21:682.

Briar, S.S., Wichman, D. & Reddy, G.V. 2016. Plant-parasitic nematode problems in organic agriculture. In: D. Nandwani (Ed). Organic farming for sustainable agriculture. Cham, Switzerland: Springer International Publishing. pp. 107-122.

Bridge, J. & Starr, J.L. 2007. Plant nematodes of agricultural importance. Academic Press, Burlington, Massachusetts.

Brito, J.A., Powers, T.O., Mullin, P.G., Inserra, R.N. & Dickson, D.W. 2004. Morphological and molecular characterization of *Meloidogyne mayaguensis* isolates from Florida. *Journal of Nematology*, 36: 232-240.

Brown, C.R., Mojtahedi, H., James, S., Novy, R.G. & Love S. 2006. Development and evaluation of potato breeding lines with introgressed resistance to Columbia root-knot nematode (*Meloidogyne chitwoodi*). *American Journal of Potato Research*, 83:18.

Brown, B., Nuberg, I. & Llewellyn, R. 2017. Negative evaluation of conservation agriculture: perspectives from African smallholder farmers. *International Journal of Agricultural Sustainability*, 15:467-481. https://doi.org/10.1080/14735903.2017.1336051.

CABI (Centre for Agriculture and Bioscience International). 2002b. *Meloidogyne incognita*. Distribution maps of plant diseases. CAB International, Wallingford, UK. Map No. 854.

CABI (Centre for Agriculture and Bioscience International). 2003. *Meloidogyne arenaria*. Distrubution maps of plant diseases. CAB International, Wallingford, UK. Map No. 900.

CABI (Centre for Agriculture and Bioscience International). 2005. Meloidogyne ethiopica. Distrubution maps of plant diseases. CAB International, Wallingford, UK. Map No. 962.

Cadet, P. & Spaull, V.W. 2003. Effect of nematodes on the sustained production of sugarcane in South Africa. *Field Crops Research,* 83: 91-100. https://doi.org/10.1080/14735903.2017.1336051.

Carneiro, R.M., Almeida, M.R. & Quénéhervé, P. 2000. Enzyme phenotypes of *Meloidogyne* spp. populations. *Nematology*, 2: 645-654.

Carneiro, R.M.D.G., de Oliveira Lima, F.S. & Correia, V.R. 2017. Methods and tools currently used for the identification of plant parasitic nematodes. In: Shah, M.M. and Mahamood, M. (Eds). Nematology-Concepts, diagnosis and control. pp. 19-35.

Carneiro, R.M.D.G., Monteiro, J.M.S., Silva, U.C. & Gomes, G. 2016. Gênero *Meloidogyne*: diagnose através de eletroforese de isoenzimas e marcadores SCAR *[Meloidogyne* genus: diagnosis through isoenzyme electrophoresis and markers SCAR]. In: Oliveira, C.M., Santos, M.A. and Castro L.H.S. (Eds). Diagnose de

Fitonematoides [Phytomatomatoid diagnosis]. Campinas, Brazil: Millennium Editora. pp. 71-93.

Carneiro, R.M.D.G., Tigano M.S., Randig O., Almeida M.R.A., and Sarah J.L. 2004a. Identification and genetic diversity of *Meloidogyne* spp. (Tylenchida:Meloidogynidae) on coffee from Brazil, Central America and Hawaii. *Nematology*, 6:287-298. https://doi.org/10.1163/1568541041217942.

Carneiro, R.M.D.G., Tigano, M.S., Randig, O., Almeida, M.R.A. & Sarah, J.L. 2004 Identification and genetic diversity of *Meloidogyne* spp. (Tylenchida: *Meloidogynidae*) on coffee from Brazil, Central America and Hawaii. *Nematology*, 6:287-298.

Cenis, J.L., Opperman, C.H. & Triantaphyllou, A.C. 1992. Cytogenetic, enzymatic and restriction fragment length polymorphism variation of *Meloidogyne* spp. from Spain. *Phytopathology*, 82: 527-531.

Chitambo, O., Haukeland, S., Fiaboe, K.K.M. & Grundler, M.W.F. 2019. African nightshade and African spinach decrease root-knot nematode and potato cyst nematode soil infestation in Kenya. *Plant Disease*, 103:1621-1630. https://doi.org/10.1094/PDIS-07-18-1193-RE.

Chitwood, D. & Perry, R.N. 2009. Reproduction, physiology and biochemistry. In: Chitwood, D.J. & Perry, R.N. (Eds). Root-knot nematodes. Wallingford, UK., CAB International Publishing. 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. North-West University, Potchefstroom, South Africa. {MSc Dissertation}.

Coolen, W.A. & D'Herde, C.J. 1972. A method for the quantitative extraction of nematodes from plant tissue. Ministry of Agriculture, Belgium. p.1- 77.

Coyne, D.L., Cortada, L., Dalzell, J.J., Claudius-Cole, A.O., Haukeland, S., Luambano, N. & Talwana, H. 2018. Plant-parasitic nematodes and food security in sub-Saharan Africa. *Annual review of phytopathology*, 56:381-403.

Curran, J., Baillie, D.L. & Webster, J.M. 1985. Use of genomic DNA restriction

fragment length differences to identify nematode species. *Parasitology*, 90:137-144. https://doi.org/10.1017/S0031182000049088.

Curran, J., McClure, M.A. & Webster, J.M. 1986. Genotypic differentiation of *Meloidogyne* populations by detection of restriction fragment length difference in total DNA. *Journal of Nematology,* 18: 83-86.

Curran, J. & Webster, J.M.1987. Identification of nematodes using restriction fragment length differences and species-specific DNA probes. *Canadian Journal of Plant Pathology,* 9:162-166. https://doi.org/10.1080/07060668709501897.

Curtis, R.H.C, Robinson, A.F. & Perry, R.N. 2009. Hatch and host location. In: Perry, R.N., Moens, M. & Starr, J.L. (Eds). Root-knot nematodes. Wallingford, UK. CAB International Publishing. pp. 139-162.

D'Addabbo, T., Sasanelli, N., Lamberti, F., Greco, P. & Carella, A. 2003. Olive pomace and chicken manure amendments for control of *Meloidogyne incognita* over two crop cycles. *Nematropica*, 33:1-7.

Dababat, A.A. & Fourie, H. 2018. Nematode parasites of cereals. In: Luc, M., Sikora, R.A. & Bridge, J. (Eds). Plant-parasitic nematodes in subtropical and tropical agriculture. Second Edition: Wallingford, UK.CAB International Publishing. pp. 131-192.

da Cunha, T.G., Visôtto, L.E., Lopes, E.A., Oliveira, C.M.G. & God, P.I.V.G. 2018. Diagnostic methods for identification of root-knot nematodes species from Brazil. *Ciência Rural*, 48:1-11. http://dx.doi.org/10.1590/0103-8478cr20170449.

DAFF (Department of Agriculture, Fisheries and Forestry). 2008. Maize production guidelines. DAFF, Pretoria, South Africa. Available at http://www.daff.gov.za/Daffweb3?Portals/0/Brochures%20and%20Production%20guidelines/Brochure%20Maize.pdf (Accessed 10 September 2021).

DAFF (Department of Agriculture, Fisheries and Forestry). 2010. Sunflower production guidelines. DAFF, Pretoria, South Africa. Available at http://www.daff.gov.za/Daffweb3?Portals/0/Brochures%20and%20Production%20guidelines/Brochure%20Sunflower.pdf (Accessed 4 July 2022).

DAFF (Department of Agriculture, Fisheries and Forestry). 2017. Trend in the agricultural sector. DAFF, Pretoria, South Africa. Available at https://www.dalrrd.gov.za/Portals/0/Statistics%20and%20Economic%20Analysis/Statistical%20Information/Trends%20in%20the%20Agricultural%20Sector%202017.pdf (Accessed 4 May 2022).

DAFF (Department of Agriculture, Fisheries and Forestry). 2018. Trends in the agricultural sector. DAFF, Pretoria, South Africa. Available at https://www.dalrrd.gov.za/Portals/0/Statistics%20and%20Economic%20Analysis/Statistical%20Information/Trends%20in%20the%20Agricultural%20Sector%202018.pdf (Accessed 14 September 2022).

DAFF (Department of Agriculture, Fisheries and Forestry). 2019. Trend in the agricultural sector. DAFF, Pretoria, South Africa. Available at https://www.dalrrd.gov.za/Portals/0/Statistics%20and%20Economic%20Analysis/Statistical%20Information/Trends%20in%20the%20Agricultural%20Sector%202019.pdf (Accessed 14 September 2022).

DAFF (Department of Agriculture, Fisheries and Forestry). 2020. Trends in the agricultural sector. DAFF, Pretoria, South Africa Available at https://www.dalrrd.gov.za/Portals/0/Statistics%20and%20Economic%20Analysis/Statistical%20Information/Trends%20in%20the%20Agricultural%20Sector%202020.pdf (Accessed 13 November 2021).

da Silva, M. do C.L., Santos, C.D.G. & da Silva, G.S. 2016. Species of *Meloidogyne* associated with vegetables in microregions of the state of Ceará. *Revista Ciência Agronômica*, 47:710-719. http://dx.doi.org/10.5935/1806-6690.20160085.

Decraemer, W. & Hunt, D.J. 2013. Structure and classification. In: Perry, R.N. & Moens, M. (Eds). Plant Nematology. Second Edition. Wallingford, UK:CAB International. pp. 3-39.

De Ponti, T., Rijk, B. & Van Ittersum, M.K. 2012. The crop yield gap between organic and conventional agriculture. *Agricultural Systems*, 108:1-9. https://doi.org/10.1016/j.agsy.2011.12.004.

De Waele, D. & Jordaan, E.M. 1988. Plant-parasitic nematodes on field crops in South Africa. 1. Maize. *Revue de Nematology Journal,* 11: 203-212.

Dhau, I., Adam, E., Mutanga, O. & Ayisi, K.K. 2018. Detecting the severity of maize streak virus infestations in maize crop using *in situ* hyperspectral data. *Transactions of the Royal Society of South Africa Journal*, 73: 8-15. https://doi.org/10.1080/0035919X.2017.1370034.

Djigal, D., Saj, S., Rabary, B., Blanchart, E. & Villenave, C. 2012. Mulch type affects soil biological functioning and crop yield of conservation agriculture systems in a long-term experiment in Madagascar. *Soil and tillage research*, 118:11-21. https://doi.org/10.1016/j.still.2011.10.008.

Dodo, M.K. 2020. Understanding Africa's food security challenges. In *Food Security in Africa*. Available at https://www.intechopen.com/chapters/71718. (Accessed 20 September 2021).

Dong, K., Dean, R.A., Fortnum, B.A. & Lewis, S.A. 2001. Development of PCR primers to identify species of root-knot nematodes: *Meloidogyne arenaria*, *M. hapla*, *M. incognita* and *M. javanica*. *Nematropica*, 31: 271-280.

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.pmpp.2018.08.004.

Du Plessis, J. 2003. Maize production. Department of Agriculture. Pretoria, South Africa. Available at http://www.arc.agric.za/arc-gci/Fact%20Sheets%20Library/Maize-infopak.pdf(Accessed 30 July 2021).

Edwards, J. H., Thurlow, D. L., & Eason, J. T. 1988. Influence of tillage and crop rotation on yields of corn, soybean, and wheat. *Agronomy Journal*, 80:76-80.

Ekpa, O., Palacios-Rojas, N., Kruseman, G., Fogliano, V. & Linnemann, A.R. 2019. sub-Saharan African maize-based foods-processing practices, challenges and opportunities. *Food Reviews International*, 35: 609-639. https://doi.org/10.1080/87559129.2019.1588290.

Engelbrecht, E. 2012. Nematode (Phylum Nematoda) community assemblages: A tool to implement environmentally-sound management strategies for root-knot nematodes (*Meloidogyne* spp.) in potato-based cropping systems. North-West University, Potchefstroom, South Africa. {MSc Thesis}.

Engelbrecht, G., Claassens, S., Mienie, C. & 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: 242. https://doi.org/10.3390/agriculture10060242.

EPPO (European and Mediterranean Plant Protection Organization). 2014. EPPO Data sheets on quarantine pests: *Meloidogyne enterolobii*. *EPPO Bulletin*, 44:159-163. https://doi.org/10.1111/epp.12120.

Esbenshade, P.R. & Triantaphyllou, A.C. 1985. Use of enzyme phenotypes for identification of *Meloidogyne* species. *Journal of Nematology*, 17:6.

Esbenshade, P.R. & Triantaphyllou, A.C. 1990. Isozyme phenotypes for the identification of *Meloidogyne* species. *Journal of Nematology*, 22:10-5.

FAO (Food and Agriculture Organisation). 2017. Food and Agriculture Organisation of the United Nations. World Food Situation: FAO cereal supply and demand brief. Available at: http://www.fao.org/wordfoodsituati-on/csdb/en/(Accessed on 13 April 2021).

FAO (Food and Agricultural Organisation). 2018a. Conservation agriculture. Available at http:// www.fao.org/conservation-agriculture/en(Accessed 19 October 2021).

Farnham, D.E., Benson, G.O. & Pearce, R.B. 2003. Corn perspective and culture. Chapter 1. In: White, P.J., Johnson, L.A. (Eds). Corn: Chemistry and technology, Edition 2nd. American Association of Cereal Chemists, Inc.st.Paul, Minesota, USA. pp. 1-33.

Fernández-Luqueño, F., López-Valdez, F., Miranda-Arámbula, M., Rosas-Morales, M., Pariona, N. & Espinoza-Zapata, R. 2014. An introduction to the sunflower crop. In: Arribas, J.I. (Eds).Sunflowers: Growth and development, environmental influences and pests/diseases. Valladolid, Spain: Nova Science Publishers. pp.1-18.

Filho, J.V. de A., Machado, A.C.Z. & Camargo, L.E.A. 2016. Root-knot nematode (Meloidogyne spp.) parasitizing resistant tobacco cultivars in Southern Brazil. *Plant Disease*, 100:1222-1231. https://doi.org/10.1094/PDIS-03-15-0341-RE.

Florini, D. A., Lorai, R. & Kotcon, J. B. 1987. Influence of edaphic factors and previous crop on *Pratylenchus* spp. population densities in potatoes. *Journal of Nematology*. 19:85-92.

Fosu-Nyarko, J. & Jones, M.G. 2016. Advances in understanding the molecular mechanisms of root-lesion nematode host interactions. *Annual Review of Phytopathology*, 54: 253-278.

Fourie, H. 2022. Maize and root-knot nematodes. In: Sikora, R.A., Desaeger, J. & Molendijk, L.P.G. (Eds). A problematic, deep-seated association. Integrated nematode management: State-of-the-art and visions for the future. Wallingford. CAB International Publishing. pp. 41

Fourie, H., Bekker, S., Mc Donald, A.H. & Engelbrecht, E. 2011. Prospective extended benefits of plant-nematode diagnostic and advisory services should closer cooperation between laboratories be attainable. Proceedings of the 20th Symposium of the Nematological Society of Southern Africa, 15-18 May 2011, Spier Estate, Stellenbosch, South Africa.

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. http://dx.doi.org/10.17159/SAJS.2015/20140212.

Fourie, H., Mc Donald, A.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. http://doi.org/10.5897/AJAR11.706.

Fourie, H., Spaull, V.W., Jones, R.K., Daneel, M.S. & De Waele, D. 2017.Introduction. 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, Switerland; Springer International Pubishing. pp.1-12.

Fourie, H., Zijlstra, C. & Mc Donald, A.H. 2001. Identification of root-knot nematode species occurring in South Africa using the SCAR - PCR technique. *Nematology*, 3:675-680.

Freitas, V.M., Silva, J.G.P., Gomes, C.B., Castro, J.M.C., Correa, V.R. & Carneiro, R.M.D.G. 2017. Host status of selected cultivated fruit crops to *Meloidogyne enterolobii*. *European Journal of Plant Pathology*, 148:307-319. https://doi.org/10.1007/s10658-016-1090-8.

Govaerts, B., Mezzalama, M., Sayre, K. D., Crossa, J., Nicol, J. M. & Deckers, J. 2006. Long-term consequences of tillage, residue management, and crop rotation on maize/wheat root rot and nematode populations. *Appl. Soil Ecol*. 32:305-315. https://doi.org/10.1016/j.apsoil.2005.07.010.

GrainSA (Grain South Africa). 2016. Results of three years' national sunflower disease surveys. Pretoria, South Africa. Available at https://www.grainsa.co.za. (Accessed 28 September 2022).

GrainSA (Grain South Africa). 2017. Sunflowers- a valuable tool in your cropping programme. Pretoria, South Africa. Available at https://www.grainsa.co.za. (Accessed 16 October 2022).

GrainSA (Grain South Africa). 2021. Control weeds early to maximise sunflower yields. Pretoria, South Africa. Available at https://www.grainsa.co.za. (Accessed 28 September 2022).

GrainSA (Grain South Africa). 2021a. Selection of the best cultivars is a difficult task. Pretoria, South Africa. Available at https://www.grainsa.co.za.(Accessed 28 September 2022).

Greco, N. & Di Vito, M. 2009. Population dynamics and damage levels. In: Perry, R.N., Moens, M. and Starr, J.L. (Eds). Root-knot nematodes. Wallingford, UK. CAB International publishing. pp. 246-269.

Haarhoff, S.J. 2020. New perspectives on plant population and row spacing of rainfed maize. Stellenbosch University, Stellenbosch, South Africa. {PhD Dissertation}.

Haarhoff, S.J., Kotzé, T.N. & Swanepoel, P.A. 2020. A prospectus for sustainability of rainfed maize production systems in South Africa. Crop Science, 60: 14-28. https://doi.org/10.1002/csc2.20103.

Hallauer, A.R., Carena, M.J. & Filho, M.J.B. 2010. Quantitative genetics in maize breeding. Springer: *Handbook of Plant Breeding*, 6:1-664.

Hernandez, A., Fargette, M. & Sarah, J.L. 2004. Characterization of *Meloidogyne* spp. (Tylenchida: Meloidogynidae) from coffee plantations in Central America and Brazil. *Nematology*, 6:193-204. https://doi.org/10.1163/1568541041217933.

Heyns, J. 1971. *A guide to plant and soil nematodes of South Africa*, (pp. 1-233). A.A Balkema, Cape Town, RSA.

Hlongwane, J.J., Ledwaba, L.J. & Belete, A. 2014. Analyzing the factors affecting the market participation of maize farmers: A case study of small-scale farmers in greater Giyani Local Municipality of the Mopani District, Limpopo Province. *African journal of agricultural research*, 9:895-899.

Holbein, J., Franke, R.B., Marhavý, P., Fujita, S., Górecka, M., Sobczak, M., Geldner, N., Schreiber, L., Grundler, F.M. & Siddique, S. 2019. Root endodermal barrier system

contributes to defence against plant-parasitic cyst and root-knot nematodes. *The Plant Journal*, 100: 221-236. https://doi.org/10.1111/tpj.14459.

Hooper, D.J., Hallmann, J. & Subbotin, S.A. 2005. Methods for extraction, processing and detection of plant and soil nematodes. In: Luc, M., Sikora, R.A. & Bridge, J. (Eds). Plant parasitic nematodes in subtropical and tropical agriculture. Second Edition: Wallingford, UK.CAB International Publishing. pp. 53-86.

Hu, M.X., Xhou, K. & Liao, J.L. 2011. Multiplex PCR for the simultaneous identification and detection of *Meloidogyne incognita*, *M. enterolobii*, and *M. javanica* using DNA extracted directly from individual galls. *Phytopathology*, https://doi.org/10.1094/phyto-04-11-0095.

Hunt, D.J. & Handoo, Z.A. 2009. Taxonomy, identification and principal species. In: Perry, R.N., Moens, M. & Starr, J.L. (Eds). Root-knot nematodes. Wallingford, UK. CAB International publishing. pp. 55-97.

IITA. 1981. Proceedings of the third research planning conference on root-knot nematodes, *Meloidogyne* spp. Ibadan, Nigeria: IITA.

Iltis, H.H. 2000. Homeotic sexual translocations and the origin of maize (*Zea mays*, Poaceae): A new look at an old problem. *Economic Botany*, 54:7-42.

Ibrahim, D., Metwaly, H. & El-Sagheer, Atef. 2021. Synergistic effect of bioagents and antioxidants against root-knot nematode, *Meloidogyne incognita* on Sunflower. *Egyptian Journal of Agronematology*, 20:140-158. https://doi.org/10.21608/EJAJ.2021.187532.

Ingham, R.E. 1994. Nematodes. *Methods of Soil Analysis: Part 2 Microbiological and Biochemical Properties*, 5:459-490.

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*, 6:1-13. https://doi.org/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. In *Phytopathology,* 97: 51-51.

Jenkins, W.R. 1964. A rapid centrifugal flotation method for separating nematodes from soil. *Plant Disease Reporter*, 48: 692.

Jones, J.T., Haegeman, A., Danchin, E.G.J., Gaur, H.S., Helder, J. & Jones, G.K, *et al.* 2013. Top 10 plant-parasitic nematodes in molecular plant pathology. *Molecular Plant Pathology Journal,* 14: 946-961. https://doi.org/10.1111/mpp.12057.

Jones, M.G.K. & Fosu-Nyarko, J. 2014. Molecular biology of root-lesion nematodes (*Pratylenchus* spp.) and their interaction with host plants. *Annals of applied biology*, 164:163-181. https://doi.org/10.1111/aab.12105.

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. https://doi.org/10.1007/s10658-019-01844-6.

Kagoda, F., 2010. Genetic studies and recurrent selection for nematode resistance in maize. KwaZulu-Natal, Pietermarizburg, South Africa. {Doctoral dissertation}.

Kagoda, F., Derera, J., Tongoona, P., Coyne, D.L. & Talwana, H.L. 2011. Grain yield and heterosis of maize hybrids under nematode infested and nematicide treated conditions. *Journal of Nematology*, 43:209-219. https://doi.org/https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3547356/.

Kagoda, F., Hearne, S., Adewuyi, O. & Coyne, D. 2015. Response of drought tolerant maize inbreds to water stress under nematode infested conditions. *Euphytica* 206:77-87. https://doi.org/10.1007/s10681-015-1474-4.

Karssen, G., Wesemael, W. & Moens, M. 2013. Root-knot nematodes. In. Perry, R.N. & Moens, M. (Eds). Plant nematology. Second Edition: Wallingford. UK. CAB International Publishing. pp. 73-108. https://doi.org/10.1079/9781780641515.0073.

Kayani, M.Z., Mukhtar, T. & Hussain, M.A. 2018. Interaction between nematode inoculum density and plant age on growth and yield of cucumber and reproduction of *Meloidogyne incognita*. *Pakistan Journal of Zoology*, 50. http://dx.doi.org/10.17582/journal.pjz/2018.50.3.897.902.

Keetch, D.P. 1989. A perspective of plant nematology in South Africa. *South African Journal of Science*, 85: 506-508.

Keetch, D.P. & Milne, D.L. 1982. The control of plant-parasitic nematodes. *Nematology in Southern Africa,* 113-129.

Khan, A., Khanzada, K.A. & Shaukat, S.S. 2019. Prevalence of plant nematodes associated with maize in Balochistan, Pakistan. *Pakistan Journal of Zoology*, 51:1975. http://dx.doi.org/10.17582/journal.pjz/2019.51.5.sc5.

Kaur, R., Brito, J.A., Dickson, D.W. & Stanley, J.D. 2006. First report of *Meloidogyne enterolobii* on Angelonia angustifolia. *Plant Disease*, 90:1113. https://doi.org/10.1094/PD-90-1113A.

Kiewnick, S., Karssen, G., Brito, J.A., Oggenfuss, M., and Frey, J.E. 2008. First report of root-knot nematode *Meloidogyne enterolobii* on tomato and cucumber in Switzerland. *Plant Disease*, 92:1370. https://doi.org/10.1094/PDIS-92-9-1370A.

Kiewnick, S., Frey, J.E. & Braun-Kiewnick, A. 2015. Development and validation of LNA-based quantitative real-time PCR assays for the detection and identification of the root-knot nematode Meloidogyne enterolobii in complex NDA backgrounds. *Phytopathology*, 105:1245-1249. https://doi.org/10.1094/phyto-12-14-0364-r.

Kleynhans, K.P.N. 1991. The root-knot nematodes of South Africa. Department of Agricultural Development, Plant Protection Research Institute, Pretoria, South Africa. *Technical Communication.* 231: 37-40.

Kleynhans, K.P.N., Van Den Berg, E., Swart, A., Marais, M. & Buckley, N.H. 1996. Plant nematodes in South Africa. Pretoria, ARC Plant Protection Unit.

Kolombia, Y.A., Karssen, G., Viaene, N., Kumar, P.L., De Sutter, N., Joos, L., Coyne, D.L., and Bert, W. 2017. Diversity of root-knot nematodes associated with tubers of

yam (Dioscorea spp.) established using isozyme analysis and mitochondrial DNA-based identification. *Journal of Nematology*, 49:177-188.

Kolombia, Y.A., Kumar, P.L., Claudius-Cole, A.O., Karssen, G., Viaene, N., Coyne, D., and Bert, W. 2016. First report of *Meloidogyne enterolobii* causing tuber 72 galling damage on white yam (Dioscorea rotundata) in Nigeria. *Plant Disease*, 100:2173-2174. https://doi.org/10.21307/jofnem-2017-063.

Koutsovoulos, G.D., Poullet, M., Elashry, A., Kozlowski, D.K., Darocha, M., Martin-Jimenez, C., Zurletto, L., Frey, J., Ahrens, C., Kiewnick, S. & Danchin, E.G.J. 2019. The polyploid genome of the mitotic parthenogenetic root-knot nematode *Meloidogyne enterolobii*. https://doi.org/10.1101/586818.

Koutsovoulos, G.D., Poullet, M., Elashry, A., Kozlowski, D.K., Sallet, E., Da Rocha, M., Perfus-Barbeoch, L., Martin-Jimenez, C., Frey, J.E., Ahrens, C.H. & Kiewnick, S. 2020. Genome assembly and annotation of *Meloidogyne enterolobii*, an emerging parthenogenetic root-knot nematode. *Scientific Data*, 7:1-13. https://doi.org/10.6084/m9.figshare.12410363.

Korayem, A.M., Youssef, M.M.A., Ahmed, M.M. & Mohamed, M.M.M. 2011. Distribution and association of plant-parasitic nematodes with some oil crops in Egypt. *Pakistan Journal of Nematology*, 29.

Kumar, Y. & Yadav, B.C. 2020. Plant-parasitic nematodes: Nature's most successful plant parasite. *International Journal and Research and Review*, 7:379-386.

Lekunze, J., Antwi, M.A & Oladele, O.I. 2011. Socio-economic constraints to sunflower production in Bojanala farming community of the North-West province, South Africa. *Life Science Journal,* 8:502-506.

Lambert, K. & Bekal, S., 2002. Introduction to plant-parasitic nematodes. *The plant Health instructor,* 10:1094-1218. https://doi.org/10.1094. PHI-I-2002-1218-01.

Lamberti, F. & Taylor. R.S.1979. Root-knot nematodes (*Meloidogyne* spp.). Academic Press, New York.

Lawn, A., Noel, G. R. & Sinclair, J. B. 1988. Plant-parasitic nematodes associated with sunflower and maize in the Republic of Zambia. *Nematropica* 18:143-154.

Lazzeri, L., Curto, G., Leoni, O. & Dallavalle, E. 2004. Effects of glucosinolates and their enzymatic hydrolysis products via myrosinase on the root-knot nematode *Meloidogyne incognita* (Kofoid et White) Chitw. *Journal of Agricultural and Food Chemistry*, *52*: 6703-6707. https://doi.org/10.1021/jf030776u.

Lima, I.M., Souza, R.M., Silva, C.P. & Carneiro, R.M.D.G. 2005. *Meloidogyne* spp. from preserved areas of Atlanticforest in the state of Rio de Janeiro, Brazil. *Nematologia Brasileira*, 29:31-38.

Liu, H., Long, H., Yan, X. & Xu, J. 2005. Species identification and host range testing of a root-knot nematode infecting guava in Hainan province. *Journal of Nanjing Agricultural University*, 28:55-59.

Long, H.B., Sun, Y.F., Bai, C. & Peng, D.L. 2015. First report of the root-knot nematode *Meloidogyne enterolobii* infecting jackfruit tree in China. *Plant Disease*, 99:1868. https://doi.org/10.1094/PDIS-04-15-0406-PDN.

Lugo, Z., Crozzoli, R., Molinari, S., Greco, N., Perichi, G. & Jiménez-Pérez, N. 2005. Isozyme patterns of Venezuelan populations of *Meloidogyne* spp. *Fitopatologia Brasileira,* 18:26-29.

Machado, A.C.Z., & Filho, J.V.de.A. 2014. Host status and phenotypic diversity of rice cultivars resistant to *Meloidogyne* species under glasshouse conditions. *Nematology*, 16:991-999. https://doi.org/10.1163/15685411-00002825.

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 Journal*, 47: 743-758. https://doi.org/10.1007/s12600-019-00769-4.

Marais, M., Swart, A., Fourie, H., Berry, S.D., Knoetze, R. & Malan, A.P. 2017. Techniques and Procedures. 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, Switerland; Springer International Pubishing.Springer: Cham. pp. 73-117.

Mbatyoti, A., Danel, 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*. https://doi.org/10.1080/15627020.2019.1679040.

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. North-West University, Potchefstroom, South Africa. {MSc Dissertation}.

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. & De Waele, D. (Eds). Nematology in South Africa: A view from the 21st Century. Cham, Switzerland: Springer International Publishing. pp. 183-200.

Mc Donald, A.H. & Nicol, J.M. 2005. Nematode parasites of cereals. In: Luc, M., Sikora, R.A. & Bridge, J. (Eds). Plant-parasitic nematodes in subtropical and tropical agriculture. Second Edition: Wallingford, UK.CAB International Publishing. pp. 131-192.

Meena, K.S., Ramyabharathi, S.A., Raguchander, T. & Jonathan, E.I. 2016. Interaction of *Meloidogyne incognita* and *Fusarium oxysporum* in carnation and physiological changes induced in plants due to the interaction. *SAARC Journal of Agriculture*, 14: 59-69. https://doi.org/10.3329/sja.v14i1.29576.

Milne, D.L. & Du Plessis, D.P. 1964. Development of *Meloidogyne javanica* (Treub.) Chit, on tobacco under fluctuating soil temperatures. *South African Journal of Agricultural Science*, 7:673-680. https://hdl.handle.net/10520/AJA05858860_338.

Mojtahedi, H., Santo, G.S., Hang, A.N. & Wilson, J.H. 1991. Suppression of root-knot nematode populations with selected rapeseed cultivars as green manure. *Journal of Nematology,* 23:170-174. https://doi.org/https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2619149/.

Montasser, S.A., Mostafa, M.A., Korayem, A.M., Anany, A.E. & Al-Baghdady, D.M. 2013. Susceptibility of nine grape cultivars to the root-knot nematode, *Meloidogyne*

incognita and Reniform nematode, *Rotylenchulus reniformis*. *Journal of Plant Protection and Pathology*, 4: 803-814.

Moens, M., Perry, R.N. & Starr, J.L. 2009. *Meloidogyne* species – a diverse group of novel and important plant parasites. In: Perry, R.N., Moens, M. & Starr, J.L. (Eds). Root-knot Nematodes. Wallingford, UK: CAB International. pp. 1-17.

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. *Journal of Nematology*, 52:1-3. https://doi.org/10.21307/jofnem-2020-005.

Mtero, F. 2012. Commercialisation, deagrarianisation and the accumulation/reproduction dynamic: Massive maize production schemes in the Eastern Cape, South Africa.

Mukhtar, T. 2018. Management of root-knot nematodes, *Meloidogyne incognita*, in tomato with two *Trichoderma* spp. *Pakistan Journal of Zoology*, 50. http://dx.doi.org/10.17582/journal.pjz/2018.50.4.sc15.

Mukthar, T., Arooi, M., Ashfaq, M. & Gulzar, A. 2017. Resistance evaluation and host status of selected green gram genotypes against *Meloidogyne incognita*. *Crop Protection Journal*, 92: 198-202.

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.

Navia, D., Delgado, A., Viera, W., Báez, F. & Trevor, J. 2017. Application of Bio-Products in Ecuadorian Agriculture: Case Banana. *International Journal of Clinical and Biological Sciences*, 2: S7.

Ngobeni, G.L., Fourie, H., Mc Donald, A.H. & Mashela, P.W. 2010. Host suitability of selected South Africa 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, 28:49-54. https://doi.org/10.1080/02571862.2011.10640012.

Ngoune Tandzi, L. & Mutengwa, C.S. 2020. Estimation of maize (*Zea mays* L.) yield per harvest area: Appropriate methods. *Agronomy*, 10: 29. https://doi.org/10.3390/agronomy10010029.

Ntidi, K. N., Daneel, M. S. & Fourie, H. 2019. The host status of *Amaranthus* spp. genotypes to *Meloidogyne* spp. and verification of resistance in genotype Thohoyandou. *Crop Protection*, 115, 64–74 https://doi.org/10.1016/j.cropro.2018.08.024.

Ntidi, K.N., Fourie, H. & Daneel, M. 2015. Greenhouse and field evaluations of commonly occurring weed species for their host suitability to *Meloidogyne* spp. *International Journal of Pest Management*, 62:11-19 https://doi.org/10.1080/09670874.2015.1087602.

Niu, J.H., Jian, H., Guo, Q.X., Chen, C.L., Wang, X.Y., Liu, Q. & Guo, Y.D. 2012. Evaluation of loop-mediated isothermal amplification (LAMP) assays based on 5S rDNA-IGS2 regions for detecting *Meloidogyne enterolobii*. *Plant Pathology*, 61:809-819. https://doi.org/10.1111/j.1365-3059.2011.02562.x.

OECD-FAO (Organisation for Economic Co-operation and Development and Food and Agriculture Organization). 2022. OECD-FAO Agricultural Outlook, OECD Agriculture statistics (database). Available at http://dx.doi.org/10.1787/agr-outl-dataen.(Accessed 2 August 2022).

Ognakossan, K.E., Mutungi, C.M., Otieno, T.O., Affognon, H.D., Sila, D.N. & Owino, W.O. 2018. Quantitative and quality losses caused by rodents in on-farm stored maize: a case study in the low land tropical zone of Kenya. *Food Security*, 10:1525-1537. https://doi.org/10.1007/s12571-018-0861-9.

Oka, Y. 2010. Mechanisms of nematode suppression by organic soil amendments: A review. *Applied Soil Ecology*, 44:101-115. https://doi.org/10.1016/j.apsoil.2009.11.003.

Oka, Y. & Yermiyahu, U. 2002. Suppressive effects of composts against the root-knot nematode *Meloidogyne javanica* on tomato. *Nematology*, 4: 891-898. https://doi.org/10.1163/156854102321122502

Oliveira, R.D.L., da Silva, M.B., da Aguiar, N.D., Bérgamo, F.L.K., da Costa, A.S.V. & Prezotti, L. 2007. The influence of parasitic nematodes on okra crop in Eastern Minas Gerais State, Brazil. *Horticultura Brasileira*, 25(1):88-93. https://doi.org/10.1590/S0102-05362007000100017.

Oliveira, C.M.G.D., Monteiro, A.R. & Blok, V.C. 2011. Morphological and molecular diagnostics for plant-parasitic nematodes: working together to get the identification done. *Tropical Plant Pathology*, 36:65-73.

Onkendi, E.M. & Moleleki, L.N. 2013. 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., Kariuki, G.M., Marais, M. & Moleleki, L.N. 2014. The threat of root-knot nematodes (*Meloidogyne* sp.) in Africa: a review. *Plant Pathology*, 63:727-737. https://doi.org/10.1111/ppa.12202.

Paes, V. dos S., Soares, P.L.M., Murakami, D.M., dos Santos, J.M., Barbosa, B.F.F., and Neves, S.S. 2012. Occurrence of *Meloidogyne enterolobii* on muricizeiro (Byrsonima cydoniifolia). *Tropical Plant Pathology*, 37:215-219. https://doi.org/10.1590/S1982-56762012000300009.

Paes-Takahashi, V. dos S, Soares, P.L.M., Carneiro, F.A., Ferreira, R.J., Almeida, E.J. de, & dos Santos, J.M. 2015. Detection of *Meloidogyne enterolobii* in mulberry seedlings (Morus nigra L.). *Ciência Rural*, 45:757-759. https://doi.org/10.1590/0103-8478cr20130350.

Parmalee, R. W. & Alston, D. G. 1986. Nematode trophic structure in conventional and no-tillage agroecosystems. *Journal of Nematology.* 18:403-407.

Papadopoulou, J. & Traintaphyllou, A.C. 1982. Sex differentiation in *Meloidogyne incognita* and anatomical evidence of sex reversal. *Journal of nematology*, 14: 549. https://doi.org/https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2618216/.

Patel, S.K. & Patel, D.J.1991. The interaction between *Meloidogyne incognita*, *M. javanica* and *Rotylenchulus Reniformis* in tobacco. *Nematologia Mediterranea*, 41-42.

Phani, V., Khan, M.R. & Dutta, T.K. 2021. Plant-parasitic nematodes as a potential threat to protected agriculture: current status and management options. *Crop Protection*, 144:105573. https://doi.org/10.1016/j.cropro.2021.105573.

Powers, T.O., Harris, T., Higgins, R., Mullin, P. & Powers, K. 2018. Discovery and identification of *Meloidogyne* species using COI DNA barcoding. *Journal of Nematology*, 50:399-412. https://doi.org/10.21307/jofnem-2018-029.

Powers, T.O., Mullin, P.G., Harris, T.S., Sutton, L.A. & Higgins, R.S. 2005. Incorporating molecular identification of *Meloidogyne* spp. into a large-scale regional nematode survey. *Journal of Nematology*, 37:226-235. https://doi.org/https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2620951/.

Pretorius, M. 2016.The abundance, identity and population dynamics of *Meloidogyne* spp. associated with maize in South Africa. North-West University, Potchefstroom, South Africa. {MSc Dissertation}.

Pretorius, M., Steenkamp, S. & Mc Donald A.H *et al*. 2014. Host status of commercial wheat and sunflower cultivars to *Meloidogyne* spp. *Journal of Nematology,* 46:223.

Putt, E.D. 1997. Early history of sunflower. In: Schneiter, A. A (Ed). Sunflower Technology and Production. Agron. Monogr. 35. ASA, CSSA, and SSSA, Madison, Wis. pp. 1-20. https://doi.org/10.2134/agronmonogr35.c1.

Quénéhervé, P., Godefroid, M., Mège, P. & Marie-Luce, S. 2011. Diversity of *Meloidogyne* spp. parasitizing plants in Martininque Island, French West Indies. *Nematropica*, 41:191-199.

Ramírez-Suárez, A., Rosas-Hernández, L., Alcasio-Rangel, S. & Powers, T.O. 2014. First report of the root-knot nematode Meloidogyne enterolobii parasitizing watermelon

from Veracruz, Mexico. *Plant Disease*, 98:428. https://doi.org/10.1094/PDIS-06-13-0636-PDN.

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*, 45:862-870. https://doi.org/10.1139/g02-054.

Randig, O., Deau, F., Santos, M.F.A. dos, Tigano, M.S., Carneiro, R.M.D.G. and Castagnone-Sereno, P. 2009. A novel species-specific satellite DNA family in the invasive root-knot nematode *Meloidogyne mayaguensis* and its potential use for diagnostics. *European Journal of Plant Pathology*, 125:485-495. https://doi.org/10.1007/s10658-009-9497-0.

Randig, O., Leroy, F., Bongiovanni, M. & Castagnone-Sereno, P. 2001. RAPD characterization of single females of the root-knot nematodes, *Meloidogyne* spp. *European Journal of Plant Pathology,* 107: 639-43. https://doi.org/10.1139/g02-054.

Ranum, P., Peña-Rosas, J. P. & Garcia-Casal, M. N. 2014. Global maize production, utilization, and consumption. *Annals of the New York Academy of Sciences*, 1312:105-112. https://doi.org/10.1111/nyas.12396.

Rashidifard, M. 2019. Comparative molecular and morphological identification, and reproduction potential of South African *Meloidogyne* species with emphasis on *Meloidogyne enterolobii*. North-West University (NWU), Potchefstroom {Thesis-PhD}.

Rashidifard, M., Fourie, H., Véronneau, P., Marais, M., Daneel, M.S. & Mimee, B. 2018. Genetic diversity and phylogeny of South African *Meloidogyne* populations using genotyping by sequencing. *Scientific Report*, 8:1-9. https://doi.org/10.1038/s41598-018-31963-9.

Rashidifard, M., Marais, M., Daneel., M.S., Mienie, C. & Fourie, H. 2019a. 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.

Rashidifard, M., Marais, M., Daneel, M.S. & Fourie, H. 2019b. 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., Fourie, H., Daneel, M.S. & Marais, M. 2019c. Morphological and morphometrical identification of *Meloidogyne* populations from various crop production areas in South Africa with emphasis on *M. enterolobii*. *Zootaxa*, 4658: 251-274. http://dx.doi.org/10.11646/zootaxa.4658.2.3.

Riekert, H.F. 1995. An adapted method for extraction of root-knot nematode eggs from maize root samples. *African Plant Protection*, 1: 41-43.

Rosa, J.M.O., Westerich, J.N. & Wilcken, S.R.S. 2013. Root-knot nematode in vegetable crops in São Paulo state, Brazil. *Nematologia Brasileira*, 37:15-19.

Sadaka, S. & Bautista, R. 2012. Grain drying tools: Equilibrium moisture content tables and psychrometric charts. University of Arkansas, Division of Agriculture Research and Extension. Retrieved from https://www.uaex.edu/publications/pdf/FSA-1074.pdf.

Sánchez-Moreno, S. & Ferris, H. 2018. Nematode ecology and soil health. In: Luc, M., Sikora, R.A. and Bridge, J. (Eds). Plant-parasitic nematodes in subtropical and tropical agriculture. Second Edition: Wallingford, UK.CAB International Publishing. pp. 62-86.

Santos, D., Abrantes, I. & Maleita, C. 2019. The quarantine root-knot nematode *Meloidogyne enterolobii* - a potential threat to Portugal and Europe. *Plant Pathology*, 68:1607-1615. https://doi.org/10.1111/ppa.13079.

Santos, M.F.A., Salgado, S.M.L., Silva, J.G.P. *et al*. 2018. *Meloidogyne incognita* parasitizing coffee plants in southern Minas Gerais, Brazil. Trop. *plant pathol*. 43, 95-98. https://doi.org/10.1007/s40858-017-0178-9.

Schoonover, J.E. & Crim, J.F. 2015. An introduction to soil concepts and the role of soils in watershed management. *Journal of Contemporary Water Research and Management*, 154: 21-47.

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. https://doi.org/10.1016/j.gfs.2020.100404.

Sharma, K. & Misra, R.S. 2011. Molecular approaches towards analyzing the viruses infecting maize (*Zea mays* L.). *Journal of General and Molecular Virology*, 3:1-17.

Shurtleff, M.C. & Averre III, C.W. 2000. Diagnosing plant diseases caused by nematodes. The American Phytopathological Society, St. Paul, Minnesota, ASP Press, p. 187.

Sibiya, J., Tongoona, P., Derera, J. & Makanda, I. 2013. Farmers' desired traits and selection criteria for maize varieties and their implications for maize breeding: A case study from KwaZulu-Natal Province, South Africa. *Journal of Agriculture and Rural Development in the Tropics and Subtropics (JARTS)*, 114:39-49.

Siddiqui, Z.A. & Mahmood, I. 1996. Biological control of plant parasitic nematodes by fungi: A review. *Bioresource Technology*, 58:229-239. https://doi.org/10.1016/S0960-8524(96)00122-8.

Signh, S., Signh, B. & Signh, A.P. 2015. Nematode: A Threat to Sustainability of Agriculture. *Procedia Environmental Sciences Journal,* 29: 215-216.

Sikora, R.A., Bridge, J. & Starr, J.L. 2005. Management practices: An overview of integrated nematode management technologies. In: Luc, M., Sikora, R.A. and Bridge, J. (Eds). Plant-parasitic nematodes in subtropical and tropical agriculture. Second Edition: Wallingford, UK. CAB International Publishing. pp. 793-825.

Silva, J.C.P.D., Medeiros, F.H.V.D. & Campos, V.P. 2018. Building soil suppressiveness against plant-parasitic nematodes. *Biocontrol Science and Technology*, 28: 423-445.

Silva, R.V. & Oliveira, R.D.L. 2010. *Meloidogyne enterolobii* (syn. *M. mayaguensis*) attacking guava in the state of Minas Gerais, Brazil. *Nematologia Brasileira*, 34:172-177.

Smale, M. & Jayne, T. 2003. Maize in eastern and southern Africa: “seeds” of success in retrospect. Environment and Production Technology Division Discussion Paper No. 97. International Food Policy Research Institute. Washington, DC. pp. 1 -90. http://www.fao.org/docs/eims/upload/166420/Smale,Jayne.pdf (Accessed 12 May 2022).

Somasundaram, J., Sinha, N.K., Dalal, R.C., Lal, R., Mohanty, M., Naorem, A.K., Hati, K.M., Chaudhary, R.S., Biswas, A.K., Patra, A.K. & Chaudhari, S.K. 2020. No-till farming and conservation agriculture in South Asia–issues, challenges, prospects and benefits. *Critical Reviews in Plant Sciences*, 39:236-279. https://doi.org/10.1080/07352689.2020.1782069.

Sorribas, F.J., Ornat, C., Verdejo-Lucas, S., Galeano, M. & Valero, J. 2005. Effectiveness and profitability of the Mi-resistant tomatoes to control root-knot nematodes. *European Journal of Plant Pathology,* 111: 29-38. https://doi.org/10.1007/s10658-004-1982-x.

Steenkamp, S. 2012. Plant-parasitiese aalwurms se impak op ‘n mielie-oes steeds onderskat. http://www.grainsa.co.za/plant-parasitiese-aalwurms-se-impak-op--n-mielie-oessteeds-onderskat (18 September 2021).

Talwana, H., Sibanda, Z., Wanjohi, W., Kimenju, W., Luambano-Nyoni, N., Massawe, C., Manzanilla-López, R.H., Davies, K.G., Hunt, D.J., Sikora, R.A. & Coyne, D.L. 2016. Agricultural nematology in East and Southern Africa: problems, management strategies and stakeholder linkages. *Pest Management Science*, 72: 226-245. https://doi.org/10.1002/ps.4104.

Tavoillot, J., Mateille, T., Ali, N., Chappé, A.M. & Martin, J.F. 2021. Early detection of the Root-knot nematode *Meloidogyne hapla* through developing a robust quantitative PCR approach compliant with the minimum information for publication of quantitative real-time PCR experiments guidelines. *Plant Disease*, 105: 2836-2843. https://doi.org/10.1094/PDIS-11-20-2408-RE.

Taylor, A.L. & Sasser, J.N .1978. Biology, identification and control of root-knot nematodes (*Meloidogyne* species). North Carolina State University and United States Agency for International Development, Raleigh. 111.

Thioulouse, J., Doledec S., Chessel D. & Olivier J. M. 1995. ADE software: multivariate analysis and graphical display of environmental data. In: Guariso, G., Rizzoli, A. Eds., Software perlambiente. Patroneditore, Bologne, pp. 5762.

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 root-knot nematode *Meloidogyne enterolobii* and development of a 88 SCAR marker for this guava-damaging species. *Plant Pathology*, 59:1054-1061. https://doi.org/10.1111/j.1365-3059.2010.02350.x.

Torres, G.R.C., Covello, V.N., Sales Júnior, R., Pedrosa, E.M.R. & Moura, R.M. 2004. *Meloidogyne mayaguensis* on *Psidium guajava* in Rio Grande do Norte. *Fitopatologia Brasileira*, 29:570. http://dx.doi.org/10.1590/S0100- 41582004000500020

Triantaphyllou, A.C. 1973. Environmental sex differentiation of nematodes in relation to pest management. *Annual Review of Phytopathology*, 11: 441-462. https://doi.org/10.1146/annurev.py.11.090173.002301.

UN (United Nations). 2022. World Population Prospects 2022: Summary of results. UN DESA/POP/2021/TR/NO. 3. United Nations, New York, USA. Available at https//www//efaidnbmnnnibpcajpcglclefindmkaj/https://www.un.org/development/desa/pd/sites/www.un.org.development.desa.pd/files/wpp2022_summary_of_results.pdf (Accessed 14 September 2022).

USDA (United State Department of Agriculture). 2018. Commodity intelligence report. Available at http://apps.fas.usda.gov/psdonline/psdHome.aspx. (Accessed 20 November 2021).

Van Zyl, K. 2013. A guide to crop pest management in South Africa, First Edition. VR Print, Pinetown.

Villar-Luna, E., Rojas-Martínes, R.I., Reyes-Trejo, B., Gómez-Rodríguez, O. & Zavaleta-Mejía, E. 2017. Mevalonate pathway genes expressed in chilli CM334 inoculated with *Phytophthora capsici* and infected by *Nacobbus aberrans* and *Meloidogyne enterolobii*. *European Journal of Plant Pathology*, 148:867-881. https://doi.org/10.1007/s10658-016-1142-0.

Visagie, M., Mienie, C.M.S. Marais, M., Daneel, M., Karssen, G. & Fourie, H. 2018. Identification of *Meloidogyne* spp. associated with agri- and horticultural crops in South Africa. *Nematology*, 20:397-401. https://doi.org/10.1163/15685411-00003160.

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

Wang, K.H., Sipes, B.S. & Schmitt, D.P. 2002. Crotalaria as a cover crop for nematode management: a review. *Nematropica*, 35-58.

Wang, Y.F., Xiao, S., Huang, Y.K., Zhou, X., Zhang, S.S. & Liu, G.K. 2014. First report of *Meloidogyne enterolobii* on carrot in China. Plant Disease, 98:1019. https://doi.org/10.1094/PDIS-02-14-0119-PDN.

Wang, Y., Wang, X.Q., Xie, Y., Dong, Y., Hu, X.Q. & Yang, Z.X. 2015. First report of Meloidogyne enterolobii on hot pepper in China. Plant Disease, 99:557-558. https://doi.org/10.1094/PDIS-08-14-0802-PDN.

Warner, F. 2009. Soil fertility, pH, texture and nematodes. Michigan State University Extension: available at https://www.canr.msu.edu/news/soil_fertility_ph_texture_and_nematodes (Accessed April 2021).

Widmer, T.L., Mitkowski, N.A. & Abawi, G.S. 2002. Soil organic matter and management of plant-parasitic nematodes. *Journal of Nematology*, 34:289. https://doi.org/https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2620584/.

Windham, G.L. & Williams, W.P. 1994. Penetration and development of Meloidogyne incognita in roots of resistant and susceptible corn genotypes. *Journal of Nematology*, 26: 80-85. https://doi.org/https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2619475/.

Xiao, S., Hou, X.Y., Cheng, M., Deng, M.X., Cheng, X. & Liu, G K. 2018. First report of Meloidogyne enterolobii on ginger (Zingiber officinale) in China. *Plant Disease*, 102:684. https://doi.org/10.1094/PDIS-09-17-1477-PDN.

Xu, J., Liu, P., Meng, Q & Long, H. 2004. Characterisation of *Meloidogyne* species from China using isozyme phenotyping and amplified mitochondrial DNA restriction fragment length polymorphism. *European Journal of Plant Pathology*, 110:309-315. https://doi.org/10.1023/B:EJPP.0000019800.47389.31.

Xue, B., Baillie D.L., Beckenbach, K. & Webster, J.M. 1992. DNA hybridisation probes for studying the affinities of three *Meloidogyne* populations. *Fundamental and Applied Nematology,* 15: 35-41.

Ye, W.M., Koenning, S.R., Zhuo, K. & Liao, J.L. 2013. First report of *Meloidogyne enterolobii* on cotton and soybean in North Carolina, United States. *Plant Disease*, 97:1262. https://doi.org/10.1094/PDIS-03-13-0228-PDN.

Yigezu Wendimu, G. 2021. Biology, taxonomy, and management of the root-knot nematode (*Meloidogyne incognita*) in sweet potato. *Advances in Agriculture*, 2021. https://doi.org/10.1155/2021/8820211.

Zijlstra, C. 2000. Identification of *Meloidogyne chitwoodi, M. fallax and M. hapla* based on SCAR-PCR: a powerful way of enabling reliable identification of populations or individuals that share common traits. *European Journal of Plant Pathology*, 106:283-290. https://doi.org/10.1023/A:1008765303364.

Zhao-hui, F. U. Chao, D. U. & W. U. Jun-xiang, W. U. 2006. Effects of Temperature, Humidity and Acidity-alkalinity on Growth and Development of *Meloidogyne incognita*.

Zondagh, S.G. & Van Rensburg, J.B.J. 1983. Progress in nematode research in maize. Technical Communication No. 182. Department of Agriculture and Fisheries, Pretoria, pp 64-66.

APPENDIX 1

Table A3.1. Fig. 3.1 *Meloidogyne* spp. numbers per 50 g roots [log(x+1) transformed for 15 localities sampled at farms of New-Era grain producers for two seasons (2019 and 2020) and two sampling intervals during each of the seasons.

Cell No.	Tukey HSD test; variable DV_1 (2 nd sampling 2019 nematode and abiotic parameter data) Homogenous Groups, alpha = ,05000 (Non-Exhaustive Search) Error: Between; Within; Pooled MS = 4625E4, df = 475,96							
	Localities	R1	DV_1 Mean	A	B	C	D	E
62	Virginia	Meloidogyne2019_2	40,3	****				
61	Virginia	Meloidogyne2019_1	87,9	****				
3	Barbespan	Meloidogyne2020_1	113,3	****				
42	Klipkop 530	Meloidogyne2019_2	115,5	****	****	****		
6	Blesbokfontein	Meloidogyne2019_2	150,3	****				
51	Lyshof	Meloidogyne2020_1	152,7	****				
17	Edenville 1	Meloidogyne2019_1	157,7	****				
55	Putfontein	Meloidogyne2020_1	168,8	****				
4	Barbespan	Meloidogyne2020_2	179,6	****				
41	Klipkop 530	Meloidogyne2019_1	181,5	****	****	****		
18	Edenville 1	Meloidogyne2019_2	209,2	****				
38	Klipkop 530	Meloidogyne2019_2	211,2	****	****	****		
37	Klipkop 530	Meloidogyne2019_1	211,2	****	****	****		
20	Edenville 1	Meloidogyne2020_2	216,9	****				
39	Klipkop 530	Meloidogyne2020_1	237,6	****	****	****		
53	Putfontein	Meloidogyne2019_1	266,6	****				
43	Klipkop 530	Meloidogyne2020_1	280,5	****	****	****		
33	Klipkop 24	Meloidogyne2019_1	293,3	****				
24	Edenville 2	Meloidogyne2020_2	299,2	****				
32	Kaalbaas	Meloidogyne2020_2	308,6	****				
2	Barbespan	Meloidogyne2019_2	311,7	****				
5	Blesbokfontein	Meloidogyne2019_1	344,7	****				
44	Klipkop 530	Meloidogyne2020_2	370,3	****	****	****		
19	Edenville 1	Meloidogyne2020_1	377,9	****				
29	Kaalbaas	Meloidogyne2019_1	381,3	****				
49	Lyshof	Meloidogyne2019_1	390,2	****				
7	Blesbokfontein	Meloidogyne2020_1	392,3	****				
50	Lyshof	Meloidogyne2019_2	399,7	****				
58	Stella	Meloidogyne2019_2	410,7	****				
36	Klipkop 24	Meloidogyne2020_2	414,6	****				
54	Putfontein	Meloidogyne2019_2	454,7	****	****			
56	Putfontein	Meloidogyne2020_2	465,9	****	****	****		
40	Klipkop 530	Meloidogyne2020_2	472,4	****	****	****		
21	Edenville 2	Meloidogyne2019_1	506,0	****	****	****		
34	Klipkop 24	Meloidogyne2019_2	517,1	****	****	****		
22	Edenville 2	Meloidogyne2019_2	535,3	****	****	****		

30	Kaalbaas	Meloidogyne2019_2	539,0	****	****	****		
57	Stella	Meloidogyne2019_1	564,7	****	****	****		
1	Barbespan	Meloidogyne2019_1	590,3	****	****	****		
46	Lareystryd	Meloidogyne2019_2	605,0	****	****	****		
35	Klipkop 24	Meloidogyne2020_1	609,8	****	****	****		
60	Stella	Meloidogyne2020_2	634,3	****	****	****		
23	Edenville 2	Meloidogyne2020_1	643,9	****	****	****		
52	Lyshof	Meloidogyne2020_2	774,8	****	****	****		
48	Lareystryd	Meloidogyne2020_2	975,3	****	****	****		
31	Kaalbaas	Meloidogyne2020_1	1047,0	****	****	****		
63	Virginia	Meloidogyne2020_1	1537,0	****	****	****		
59	Stella	Meloidogyne2020_1	1618,6	****	****	****		
14	Dwaalkraal	Meloidogyne2019_2	1646,3	****	****	****		
47	Lareystryd	Meloidogyne2020_1	1650,0	****	****	****		
45	Lareystryd	Meloidogyne2019_1	1948,8	****	****	****		
8	Blesbokfontein	Meloidogyne2020_2	2200,0	****	****	****		
13	Dwaalkraal	Meloidogyne2019_1	3274,3	****	****	****		
64	Virginia	Meloidogyne2020_2	4413,0	****	****	****		
25	Hoopstad	Meloidogyne2019_1	4483,4	****	****	****		
15	Dwaalkraal	Meloidogyne2020_1	5243,7	****	****	****		
26	Hoopstad	Meloidogyne2019_2	6384,9	****	****	****		
10	Bothania	Meloidogyne2019_2	6643,0	****	****	****		
11	Bothania	Meloidogyne2020_1	7268,6	****	****	****		
9	Bothania	Meloidogyne2019_1	8099,0	****	****	****		
27	Hoopstad	Meloidogyne2020_1	13585,6		****	****		
12	Bothania	Meloidogyne2020_2	13626,1			****		
16	Dwaalkraal	Meloidogyne2020_2	38462,9				****	
28	Hoopstad	Meloidogyne2020_2	110344,0					****

Table A3.1. Fig. 3.1 *Rotylenchulus* spp. numbers per 50 g roots [log(x+1) transformed for 15 localities sampled at farms of New-Era grain producers for two seasons (2019 and 2020) and two sampling intervals during each of the seasons.

Cell No.	Tukey HSD test; variable DV_1 (2 nd sampling 2019 nematode and abiotic parameter data) Homogenous Groups, alpha = ,05000 (Non-Exhaustive Search) Error: Between; Within; Pooled MS = 8751E2, df = 475,99					
	Localities	R1	DV_1 Mean	A	B	C
62	Virginia	Rotylenchulus2019_2	23,222	****		
6	Blesbokfontein	Rotylenchulus2019_2	25,667	****		
3	Barbespan	Rotylenchulus2020_1	40,444	****		
20	Edenville 1	Rotylenchulus2020_2	47,778	****		
18	Edenville 1	Rotylenchulus2019_2	51,333	****		
38	Klipkop 530	Rotylenchulus2019_2	52,800	****	****	
4	Barbespan	Rotylenchulus2020_2	58,667	****		

58	Stella	Rotylenchulus2019_2	62,333	****		
19	Edenville 1	Rotylenchulus2020_1	62,889	****		
35	Klipkop 24	Rotylenchulus2020_1	63,556	****		
44	Klipkop 530	Rotylenchulus2020_2	66,000	****	****	
33	Klipkop 24	Rotylenchulus2019_1	69,222	****		
42	Klipkop 530	Rotylenchulus2019_2	74,250	****	****	
2	Barbespan	Rotylenchulus2019_2	77,000	****		
30	Kaalbaas	Rotylenchulus2019_2	80,667	****		
34	Klipkop 24	Rotylenchulus2019_2	84,333	****		
50	Lyshof	Rotylenchulus2019_2	84,333	****		
36	Klipkop 24	Rotylenchulus2020_2	84,667	****		
23	Edenville 2	Rotylenchulus2020_1	84,889	****		
7	Blesbokfontein	Rotylenchulus2020_1	88,000	****		
52	Lyshof	Rotylenchulus2020_2	88,000	****		
46	Lareystryd	Rotylenchulus2019_2	91,667	****		
56	Putfontein	Rotylenchulus2020_2	93,333	****		
55	Putfontein	Rotylenchulus2020_1	94,444	****		
40	Klipkop 530	Rotylenchulus2020_2	94,600	****	****	
37	Klipkop 530	Rotylenchulus2019_1	99,000	****	****	
39	Klipkop 530	Rotylenchulus2020_1	116,600	****	****	
17	Edenville 1	Rotylenchulus2019_1	118,333	****		
41	Klipkop 530	Rotylenchulus2019_1	124,500	****	****	
24	Edenville 2	Rotylenchulus2020_2	125,333	****		
64	Virginia	Rotylenchulus2020_2	126,222	****		
63	Virginia	Rotylenchulus2020_1	128,667	****		
29	Kaalbaas	Rotylenchulus2019_1	143,000	****		
11	Bothania	Rotylenchulus2020_1	151,333	****		
61	Virginia	Rotylenchulus2019_1	153,444	****		
51	Lyshof	Rotylenchulus2020_1	183,000	****		
47	Lareystryd	Rotylenchulus2020_1	188,333	****		
43	Klipkop 530	Rotylenchulus2020_1	198,000	****	****	
31	Kaalbaas	Rotylenchulus2020_1	205,111	****	****	
14	Dwaalkraal	Rotylenchulus2019_2	220,000	****	****	
8	Blesbokfontein	Rotylenchulus2020_2	243,000	****	****	
21	Edenville 2	Rotylenchulus2019_1	264,000	****	****	
60	Stella	Rotylenchulus2020_2	267,222	****	****	
12	Bothania	Rotylenchulus2020_2	289,444	****	****	
48	Lareystryd	Rotylenchulus2020_2	300,667	****	****	
53	Putfontein	Rotylenchulus2019_1	318,222	****	****	
54	Putfontein	Rotylenchulus2019_2	341,000	****	****	
22	Edenville 2	Rotylenchulus2019_2	352,000	****	****	
1	Barbespan	Rotylenchulus2019_1	377,667	****	****	
49	Lyshof	Rotylenchulus2019_1	451,444	****	****	
57	Stella	Rotylenchulus2019_1	462,000	****	****	
59	Stella	Rotylenchulus2020_1	493,667	****	****	

32	Kaalbaas	Rotylenchulus2020_2	500,111	****	****	
5	Blesbokfontein	Rotylenchulus2019_1	531,556	****	****	
26	Hoopstad	Rotylenchulus2019_2	552,222	****	****	
10	Bothania	Rotylenchulus2019_2	584,333	****	****	
27	Hoopstad	Rotylenchulus2020_1	594,333	****	****	
13	Dwaalkraal	Rotylenchulus2019_1	597,667	****	****	
25	Hoopstad	Rotylenchulus2019_1	621,444	****	****	
15	Dwaalkraal	Rotylenchulus2020_1	1024,889	****	****	
9	Bothania	Rotylenchulus2019_1	1134,000	****	****	
45	Lareystryd	Rotylenchulus2019_1	1181,667	****	****	
28	Hoopstad	Rotylenchulus2020_2	2007,667		****	
16	Dwaalkraal	Rotylenchulus2020_2	6368,333			****