The abundance, identity and population dynamics of *Meloidogyne* spp. associated with maize in South Africa

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ABSTRACT

Maize, a staple food in sub-Saharan countries, is also an important livestock feed source in many parts of the world. Plant-parasitic nematodes cause estimated yield losses of 12 % upwards in local maize fields. Although *Pratylenchus* spp. were initially regarded as the economically most important plant-parasitic nematodes of maize. However, improving the efficacy of a NaOCl-method for extraction of *Meloidogyne* spp. from maize roots during 1995 proved otherwise. *Meloidogyne incognita* and *M. javanica* were from then on listed as the predominant species that adversely affected local maize production, followed by *Pratylenchus zeae*.

The objectives of this study were to i) conduct a follow-up audit of nematode pests of maize in South Africa and ii) determine the nature of relationships between initial (Pi) and final population densities (Pf) of *M. incognita* and *M. javanica,* respectively, in microplot experiments as well as the effect of a seed treatment (active substance: abamectin) on Pi levels of a mixed *M. incognita* and *M. javanica* (70:30 ratio) population.

Root and soil samples were obtained from 78 commercial maize fields (irrigation and rain-fed) in local maize-production areas during the 2014/15 and 2015/16 growing seasons. Plant-parasitic nematodes were extracted from the samples using standard protocols, counted and identified. Molecular species identification was done, for *Meloidogyne* only, using the sequence-characterised amplified region (SCAR) - polymerase chain reaction (PCR) and NaDH5-gene sequencing. *Meloidogyne incognita,* followed by *Meloidogyne javanica*, *Meloidogyne arenaria* and *Meloidogyne enterolobii* were the predominant root-knot nematodes identified. *Meloidogyne enterolobii* is a first report for local maize, listed as a non- or poor host crop. Crops locally used in rotation with maize are, however, highly susceptible to this species and will allow high build-ups that will be difficult to manage. Use of the NADH5 technique was not able to discriminate among the four species, as was obtained by SCAR-PCR, but grouped them in one clade with numerous thermophilic *Meloidogyne* spp. (sequences selected from NCBI Genbank). The NADH5 could also not indicate the presence of mixed species (31 % of the populations identified) as was obtained with SCAR-PCR.

Substantial variation existed among the Pf levels of *M. incognita* and *M. javanica* for different Pi levels used. The Pf of *M. incognita* at the highest Pf level (10 000 eggs and J2 / root system) was substantially higher compared to that of *M. javanica*. A sharp decline was

recorded for the production potential of *M. javanica* compared to that of *M. incognita* at Pi levels 7 500 and 10 000. Furthermore, substantial reductions of 2.2 (micro plot study) and 2.5 times (glasshouse study) were recorded in *Meloidogyne* spp. Pf densities for the abamectin compared to control treatment. Lower Pfs illustrated the benefits this environmental sustainable and cost-effective treatment may offer to local producers in reducing *Meloidogyne* spp. numbers. Identification of a more aggressive, threat species, *M. enterolobii* for which maize has been known to be a poor host, was found in a maize field in Mpumalanga. This study generated new information that will be useful for producers, related crop as well as seed and chemical industries. It accentuates the need to re-assess nematode assemblages in crop fields and exploitation of alternative and sustainable management tools to combat nematode pests.

Keywords: Damage threshold, maize, *Meloidogyne* spp., molecular characterisation, plant-parasitic nematodes, root-knot nematodes.

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A re-assessment of plant-parasitic nematodes associated with maize, with emphasis on *Meloidogyne*

Chapter 1: General introduction and literature review

1.1 General Introduction

White maize is the staple food for humans in South Africa and various other developing countries, while yellow maize is used especially as feed for livestock. Except for various diseases infesting and damaging maize, a wide range of pests also has an adverse impact on the production of the crop. Plant-parasitic nematodes constitute a pest that threatens production of maize worldwide. Root-knot nematodes, members of the genus *Meloidogyne* Goeldi, 1892 in particular, are one of the major biotic constraints that hamper local maize production. This study had a dual purpose and focused on i) generating knowledge about the abundance, occurrence and the identification of *Meloidogyne* spp. that prevail in maize fields in various production areas, ii) also addressed the effect of initial population densities on the population development of *Meloidogyne* spp. The author first introduces the reader to the origin, morphology, production practices and importance of maize by means of a concise summary. Then follows abbreviated information about the morphology, physiology, distribution and management of nematode pests with the main focus on the genus *Meloidogyne*. The next part of this chapter accentuates the different approaches (morphology, morphometric and molecular) used to identify *Meloidogyne* spp., with emphasis on the use of molecular methods and phylogeny. Following the literature review is the two technical chapters for which information was generated on i) the abundance and occurrence of *Meloidogyne* spp. extracted, counted and identified from 78 maize fields located in five provinces and ii),the reproduction potential of *Meloidogyne incognita* (Kofoid & White, 1919) Chitwood, 1949 and *Meloidogyne javanica* (Treub, 1885) Chitwood, 1949 (the two dominant root-knot nematode species identified in the second chapter) in roots of a susceptible maize cultivar using a range of initial inoculation densities (Pi) in separate microplot studies over a consecutive, two-year period. The latter study also included experiments to determine the effect of a nematicidal seed treatment on *Meloidogyne* spp. population development. The dissertation concludes with a concise overview of the major findings and how the way forward is anticipated in terms of results obtained from this study. Some results generated during this study add novel information to the knowledge base of researchers, industries and producers.

1.2 Literature review

Maize (*Zea mays* L.) is one of the most important grain crops planted annually in various countries and the most important summer crop grown in South Africa (Sibiya *et al*., 2013). Maize serves as the main food source for humans and is also a major feed grain for livestock (DAFF, 2014). World maize production reached a mean of 933 985 metric tonnes (MT) over a five-year period (2010 to 2014), with Asia being the biggest producer (Table 1.1). South Africa is generally the ninth biggest producer of maize worldwide, with the United States of America, China and Brazil being the top-three maize-producing countries (FAO, 2016).

Table 1.1 Maize production (1000 t) in various continents during 2010 to 2014 (FAO, 2016).

Continent	**Maize produced (1000 t)**
World (total)	933 985
Africa	70 162
Asia	284 440
Australia	616
Europe	107 979
North and Central America	361 513
South America	108 520

Other grain crops used as rotation crops with maize during the summer growing season of 2015/16 by South African producers, *viz*. sunflower, soybean, sorghum and groundnut, respectively, followed maize in terms of production figures (Table 1.2) (Steenkamp, 2012).

Table 1.2 Production figures for maize and other grain crops grown in South Africa during the summer-growing season of 2015/16(GrainSA, 2017).

Crop	Tonnes harvested	Hectares planted
Maize (total for white and yellow)	15 631 000	19 468 000
Sunflower Seed	755 000	718 500
Soybean	742 000	502 800
Sorghum	70 500	48 500
Groundnut	17 680	22 600

In South Africa, the maize production areas are mainly based on climatic conditions (Fig. 1.1). Of the two types of maize grown annually in South Africa, yellow maize constitute 52 % of the total area planted and is mostly used for animal feed production. The remaining 48 % of hectares is planted with white maize, which is mostly used for human consumption (DAFF, 2014).

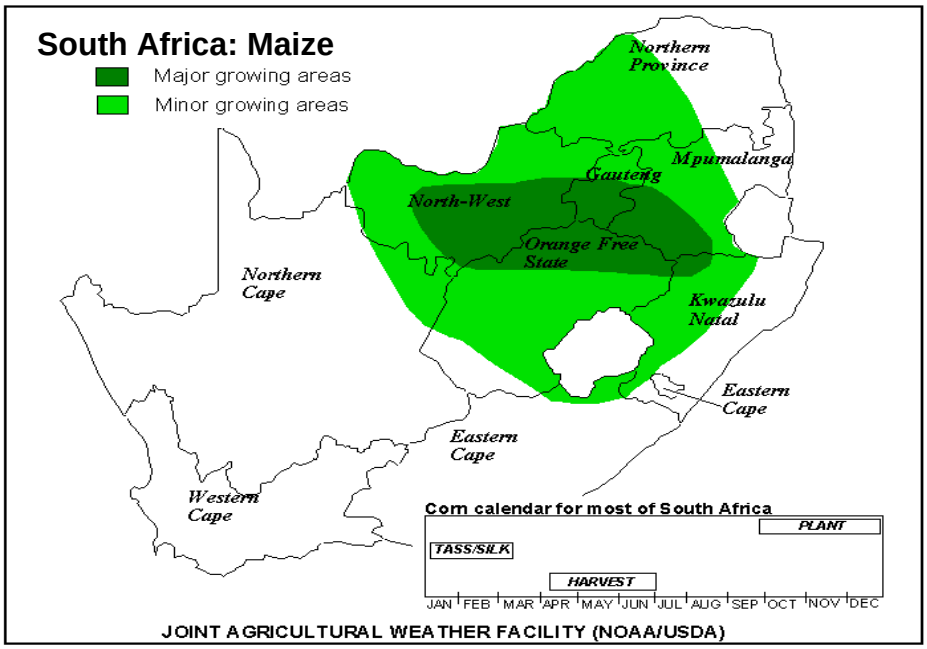


Figure.1.1 The maize production areas of South Africa (AGWEB, 2015).

1.2.1 *Zea mays* L.

1.2.1.1 Origin and history

The closest wild ancestors of maize are that of the teosinte group, which is native to Mexico and Central America (Doebley, 2004). The cultivation of maize started approximately 7 000 to 10 000 years ago in the Americas (Hallauer *et al.*, 2010). From there it has been distributed to the east and further throughout the world, arriving in Africa during the 16th century (Smale & Jayne, 2003).

1.2.1.2 Classification

Maize consist of monocotyledon plants and belongs to the Family Poaceae (Table 1.3). They are unique to the grass family because the male and female flowers are borne on the same plant as separate inflorescences on the maize ear (female inflorescences) and the tassel (male inflorescences) (Du Plessis, 2003). The genus contain five species, with only *Zea mays* cultivated throughout the world. The other species represent wild grasses called teosintes (Flannery & Piperno, 2001).

Table 1.3 The following table contain the classification of maize (CABI, 2017)

Common Name:	Maize
Super Kingdom:	Eukaryota
Kingdom:	Plantae
Phylum:	Spermatophyta
Sub Phylum:	Angiospermae
Class:	Monocotyledonae
Order:	Cyperales
Family:	Poaceae
Genus:	*Zea*
Species:	*Z. mays*

1.2.1.3 Basic morphology, growth and development

The maize kernel contains an embryo that gives rise to the next generation/crop. Kernels are also utilized as a source of carbohydrates in human food or animal feed. Germination of a maize kernel can range from two to eight days after planting depending on the soil moisture, soil temperature and the type of maize cultivar planted. Mature maize plants have profusely branched and fine root systems, which can extent downwards into the soil for up to 2 m and laterally for up to 1.5 m (Du Plessis, 2003).

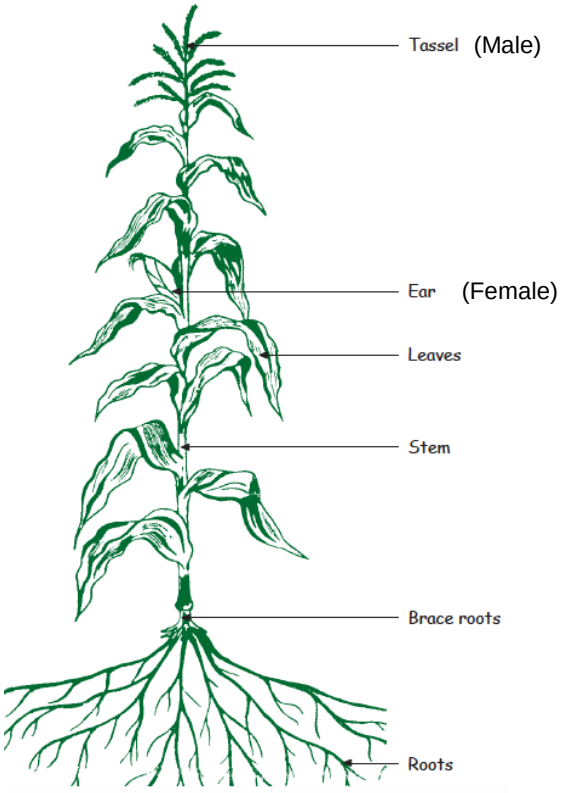


Figure 1.2 The basic morphology of a mature maize plant (Du Plessis, 2003).

Maize plants can reach heights up to 4 m (Du Plessis, 2003).The stem of a maize plant is divided into nodes and internodes and generally contains eight to 20 leaves (Fig. 1.2) that are arranged spirally and alternately in two opposite rows. The inter-florescence of maize plants are separated on each plant (Fig. 1.2). A maize ear is generally receptive to pollen for approximately three weeks but starts to decline in effectiveness after 10 days (Du Plessis, 2003).

From planting of a maize kernel (which represents Growth Stage 0) onwards, the plant follows through different growth stages until it reaches physiological maturity (Growth Stage 7). The different growth stages of maize are illustrated in Fig. 1.3.

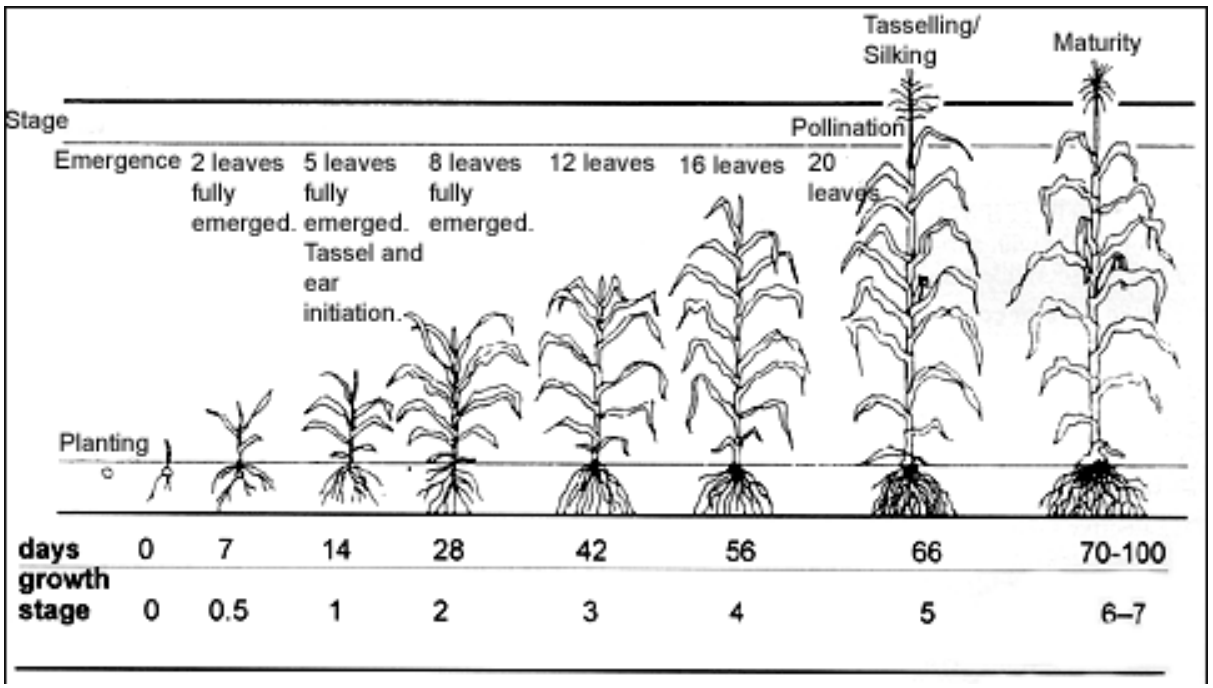


Figure 1.3 The growth stages of maize from planting to physiological maturity (Beckingham, 2007).

1.2.1.4 Adaptation, production potential and practices

1.2.1.4.1 Temperature

Maize is a summer crop and is grown in warm climates where the mean daily temperatures of the soil is not less than 10 ºC and do not exceed 31 ºC. Flowering occurs best at 19 ºC to 25 ºC. The minimum soil temperature needed for germination is 10 ºC, with the optimum range at 16 ºC to 18 ºC. Seedlings will emerge within five to six days at 20 ºC. The critical temperature that adversely affect yield is approximately 32 ºC. A frost-free period of 120 to 140 days is required to prevent damage in maize crops. Mature plants are easily damaged by frost, which affects grain filling adversely (Du Plessis, 2003; DAFF, 2008).

1.2.1.4.2 Moisture

In South Africa, more than 80 % of maize crops are produced under rain-fed conditions (where rainfall is the only source of water), with slightly less than 20 % being cultivated under irrigation (where maize fields receive rain and additional water from nearby water sources such as dams or rivers). For optimal growth and yield, maize requires an annual precipitation of 500 to 750 mm or more. Water deficiency is usually the major yield-limiting factor in local maize production, especially in rain-fed areas. Between 350 and 450 mm of rain is required per annum to obtain maize yields of 3 152 kg/ha (Du Plessis, 2003; DAFF, 2008). The total yields produced by maize on irrigated fields are more than four times higher than those produced by maize on a rain-fed field (Du Plessis, 2003).

1.2.1.4.3 Soil type and soil management practices

In South Africa, large-scale production of maize are practiced in sandy soils (with a clay content of less than 10 %) and in clay and clay-loam soils (with a clay content in excess of 30 %). Soils most suitable and favourable for maize cultivation have good and effective depth, proper internal drainage, optimal moisture regimes and sufficient and balanced quantities of plant nutrients and chemical properties (Du Plessis, 2003; DAFF, 2008).

Soil tillage is one of the biggest cost factors in maize production. Various tillage systems are currently practiced in South Africa. Conventional tillage was the most common practice used in the past, but recently farmers started to introduce more sustainable conservation agriculture practices such as no-till, minimum till, cover and stubble mulching (Du Plessis, 2003; DAFF, 2008; Esmeraldo, 2017).

Traditionally, conventional tillage practices was the most used practice that aims to control weeds, reduce water- and wind erosion and to mix the organic matter that was left behind from the previous seasons' crops into the soil to improve the soil structure (Du Toit, 1997). Conservation tillage practices involve minimum disturbance of the soil biota, soil coverage and include crop rotation to increase population levels and diversity of the beneficial soilborne organisms present. These beneficial organisms improve the soil quality and restore nutrients that were depleted through long-term conventional tillage and poor soil management of certain agricultural areas (FAO, 2008).

1.2.1.4.4 Planting date and depth

Planting commences as soon as soil moisture and temperature levels are suitable for proper germination of maize seeds. A minimum air temperature of 10 to 15 ºC must be maintained for at least seven successive days for effective germination. No germination will take place below 10 ºC. Generally, for South African climatic conditions, the optimal dates for the planting of maize range from the start of October to the first week of November (cooler eastern producing areas), or from the last week in October to mid-November (central regions) and from the last two weeks in November to mid-December (drier western areas) (Fig. 1.1). Planting depth of maize seeds varies from 5 to 10 cm, depending on the soil type and planting date. Generally, planting should be shallower in heavier soils than in sandy soils. Early plantings can be shallower, however. Plant population of a maize crop is dependent on the row width and intra-row spacing within a specific field, which in turn is depended on the mechanical equipment and the kind of tillage preparation used (Du Plessis, 2003; DAFF, 2008).

1.2.1.4.5 Cultivar choice

Various internally homogenous rainfall regions exist locally that display different precipitation levels (Thomas *et al.,* 2007). For each of these precipitation areas, numerous maize cultivars are registered that are adapted for that specific area. In selection of a cultivar, several important characteristics need to be considered such as yield potential, adaptability to a given production area and production conditions, stability to yield at a specific potential, length of the growing season, lodging, tillage method used, prolificacy, percentage grain moisture as well as disease and pest resistance of different cultivars. Informed choices made during the selection of suitable cultivars constitute a crucial part of planning and will reduce the risks producers face during a growing season (Du Plessis, 2003; DAFF, 2008).

1.2.1.4.6 Fertilisation

Local producers are advised to follow the guidelines for optimal fertilization as available in the ‘Fertilizer Guidelines for Maize’ of the Grain Crops Institute of the Agricultural Research Council, Potchefstroom. To ensure optimal fertilization of a maize crop before planting and during the growing season, soil samples should be taken from the designated field following standard procedures and submitted for laboratory analysis. Nitrogen (N), phosphorous (P) and potassium (K) are the key elements applied by producers using a fertilizer mixture. It is advised that N and K applications should not exceed 70, 50 and 30 kg/ha for 0.9, 1.5 and

2.1 m row widths, respectively, but larger quantities can be applied provided that it is placed 70 to 100 mm next to and below the seeds (Du Plessis, 2003; DAFF, 2008).

Timely and effective weed control is crucial to ensure optimal yield. Weeds compete vigorously with maize plants for nutrients and water, especially during the first six to eight weeks after planting, causing an annual yield loss of approximately 10 % for most producers. The presence of weeds during harvest may furthermore slow down the harvesting process, pollute grain seeds and result in downgrading of consignments, transmit odours to grain or incur additional costs to remove weed seeds. Moreover, seeds of certain weeds such as thorn apple (*Datura*) may be poisonous when consumed by animals or humans. Weeds are also intermediate/alternative hosts of nematode pests and hence from this viewpoint pose another constraint to producers (Du Plessis, 2003; DAFF, 2008; Ntidi *et al*., 2015).

1.2.1.4. Harvesting

Conventional producers harvest maize mechanically when the seeds obtain moisture levels of 12 to 14 %. The consignment is then delivered to the nearest silo. Developing producers generally harvest their small maize fields by hand (Du Plessis, 2003).

1.2.1.5 Production constraints

Apart from the abiotic constraints discussed above, a range of diseases and pests also represent major constraints for local producers (Sibiya *et al*., 2013). The presence and predictability of diseases and pests in maize fields vary because of the diverse climates and rainfall patterns that prevail in the different maize-producing areas (Sibiya *et al.,* 2013). Diseases and pests cause a substantial decrease in maize yields and include major fungal diseases (e.g. rust; *Puccinia sorghi*, Schwein and *P. polysora*, Underw.; grey leaf spot (*Cercospora zeae-maydis*, Thelon and Daniels) and viral diseases (e.g. maize streak virus of the MSV-A strain; family *Geminiviridae*, genus *Mastrevirus*) (Varsani, *et al*., 2008; Sibiya *et al.,* 2013). Other pests that cause significant damage to maize include insects such as the stalk borers *Chilo* partellus, Swinhoe, *Busseola fusca*, Fuller (Bate & van Rensburg, 1992), and the fall army worm *Spodoptera frugiperda*, Smith (Goergen *et al*., 2016) (as well as plant-parasitic nematodes (*Meloidogyne* and *Pratylenchus* being predominant) (Mc Donald *et al*., 2017). The next part of this chapter provides a concise overview of these nematodes, with emphasis on the economically important nematode pests that hamper local maize production.

1.2.2 Nematodes

1.2.2.1 General classification

Nematodes are microscopic, aquatic animals present in every ecological niche on earth. Nematodes are part of the Phylum Nematoda Potts, 1932 (Fig. 1.4) and roundworms are one of the oldest and most diverse phylums in the Animal Kingdom (Decraemer & Hunt, 2013). Except for plant-parasitic nematodes, those that parasitise animals (invertebrates and vertebrates) including humans had been identfied (Hickman *et al.,* 2006). Soil nematode communities include both plant-parasitic and non-parasitic or free-living nematodes such as bacterivores (feed on bacteria and other prokaryotic food substrates), fungivores (feed on fungi), omnivorous (feed on more than one food source) and predacious nematodes (feed on other micro-organisms) (Yeates *et al*., 1993).Plant-parasitic nematodes are included in the classes Enoplea Inglis, 1983 and Chromadorea Inglis, 1983, and three major orders namely Dorylaimida Pearse, 1942, Triplonchida Cobb, 1920 and Rhabditida Chitwood, 1933 (Decraemer & Hunt, 2013).

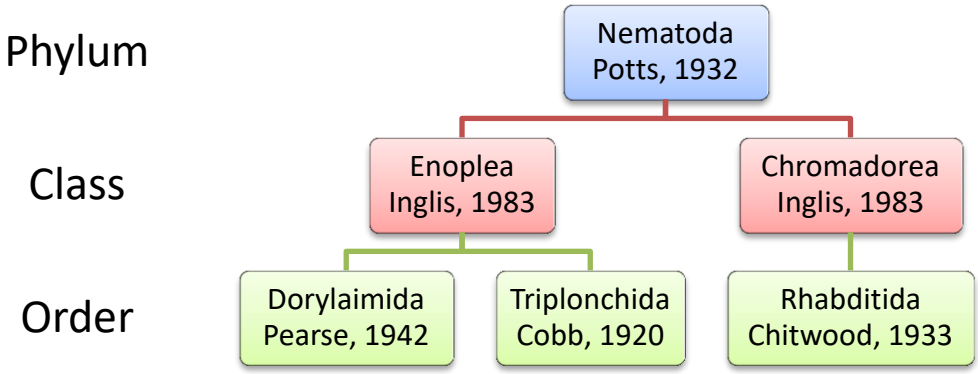


Figure 1. 4 The classification of plant-parasitic nematodes to order level as listed by Decraemer & Hunt (2013) according to the classification systems De Ley and Blaxter (2002), Siddiqi (2000) and Hunt (1993, 2008).

Since this study is primarily focused on agriculture, the emphasis will be on *Meloidogyne* spp. and, to a lesser extent, on *Pratylenchus* spp. These two genera are the economically most important and prevalent plant-parasitic nematodes that occur in soils where maize is produced in South Africa.

1.2.2.2 Basic biology and morphology of nematodes

Plant-parasitic nematodes are motile, mostly vermiform organisms that are between 0.2 to 12 mm long and range from 10 to 35 µm in diameter (Kleynhans *et al*., 1996; Colombo *et al*., 2014). In some genera such as *Meloidogyne* spp., sexual dimorphism occurs where the females are swollen and saccate-like. The females of *Meloidogyne* are thus sedentary endoparasites.

The body of a nematode is covered by a transparent cuticle with annules/striae. Plant-parasitic nematodes penetrate the cell walls of the host tissue with a stylet located in the anterior end (head) of their bodies. There are three stylet types in plant-parasitic nematodes namely a stomatostylet (Tylenchida, to which *Meloidogyne* belongs), an onchiostylet (Triplonchida) and an odontostylet (Dorylaimida).

1.2.2.3 Plant-parasitic nematodes associated with maize

Due to the recent withdrawal of some nematicides that reduced plant-parasitic nematode populations in agricultural fields, the abundance of such pests increased (Onkendi *et al.*, 2014). A survey done by Jones *et al*. (2013) listed the top ten plant-parasitic species, with *Meloidogyne* spp. being rated as the most economical important (Onkendi *et al.*, 2014). However, symptoms inflicted by plant-parasitic nematodes can be asymptomatic or similar to those caused by other soil-borne pathogens (e.g. bacteria and fungi) as well as abiotic factors (e.g. drought or flooding, when plants are deprived of nutrients) (Mitkowski & Abawi, 2003, Coyne *et al.*, 2014). Therefore, some producers are still unaware that plant parasitic nematodes cause damage to their maize crops.

Plant-parasitic nematode complexes consist of numerous species that infect and parasitize plant parts such as roots, tubers, rhizomes, pods and pegs. The most damaging nematode pests associated with maize globally include root-knot (*Meloidogyne* spp.), lesion (*Pratylenchus* spp.) and cyst (*Heterodera* spp.) nematodes (Jones *et al*., 2013). Annual yield losses caused by nematode pests in South Africa range between 12 % (Keetch, 1989) and 60 % (Riekert & Henshaw, 1998). Plant-parasitic nematodes[1] associated with maize crops locally, are listed in Table 1.4 (De Waele & Jordaan, 1988; Keetch & Buckley, 1984; Kleynhans *et al*., 1996; SAPPNS[1]).

[1]Dr Mariette Marais of the Nematology Unit, Biosystematics Division, Agricultural Research Council – Plant Health and Protection is thanked for the use of data from the South African Plant-Parasitic Nematode Survey (SAPPNS) database; E-mail: maraism@arc.agric.za

Individuals of ectoparasitic nematode genera such as *Criconema* Hofmänner and Menzel, 1914, *Criconemoides* Taylor, 1936, *Hemicriconemoides* Chitwood and Birchfield, 1957, *Hemicycliophora* de Man, 1921, *Nanidorus* Siddiqi, 1974, *Paratrichodorus* Siddiqi, 1974, *Quinisulcus* Siddiqi, 1971, *Telotylenchus* Siddiqi, 1960, *Tylenchorhynchus* Cobb, 1913 and *Xiphinema* Cobb, 1913 and semi-endoparasitic nematodes such as *Rotylenchulus* Fillipjev, 1936 are also listed, but their presence in soil samples from maize fields locally, is sporadic rather than common.

The two economically most important plant-parasitic nematode genera associated with maize crops though, are *Meloidogyne* spp. and *Pratylenchus* spp. (Mc Donald *et al*., 2017). Although *Pratylenchus* spp. were regarded as the economically most important plant-parasitic nematodes of maize during the 1970s and 1980s, improvement of the efficacy of a NaOCl-method for the extraction of root-knot nematodes (*Meloidogyne* spp.) from maize roots during the mid-1990s, proved otherwise (Riekert, 1995). The latter intervention indicated that *M. incognita* and *M. javanica* were the most common and predominant species that has the highest damage potential in maize (Riekert, 1996). These two root-knot nematode species have a high damage potential due to their high population levels and wide host ranges (Mc Donald *et al.,* 2017). Therefore, this chapter will focus hence further on *Meloidogyne* spp. with only limited reference to *Pratylenchus* spp.

Table 1.4 Plant-parasitic nematodes associated with maize in South Africa (De Waele & Jordaan, 1988; Jordaan *et al.,* 1989; Keetch & Buckley, 1984; `Kleynhans *et al*., 1996; SAPPNS[1]; Unpublished data)

Genus	Species	Genus	Species	Genus	Species	Genus	Species
Criconema	*C. anans*	*Hoplolaimus*	*H. pararobustus*	*Rotylenchulus*	*R. borealis*	*Xiphinema*	*X. bolandium*
	C. carolinae		*Hoplolaimus* sp.		*R. clavicaudatus*		*X. bourkei*
	C. lefodium	*Longidorus*	*L. africanus*		*R. leptus*		*X. capriviense*
	C. mumdum		*L. monile*		*R. parvus*		*X. dracomontanum*
	C. mutabile		*L. pisi*		*R. sacchari*		*X. elongatum*
	Criconema sp.		*Longidorus* sp.		*Rotylenchulus* sp.		*X. limpopoensis*
	C. zeae	*Meloidogyne*	*M. arenaria*	*Rotylenchus*	*R. brevicaudatus*		*X. malutiensis*
Criconemoides	*C. curvatus*		*M. hapla*		*R. capensis*		*X. mampara*
	C. ferniae		*M. incognita*		*R. devonensis*		*X. mampara f. bisexuale*
	C. obtusicaudatus		*M. javanica*		*R. gracilidens*		*X. mampara f. major*
	C. parvus		*Meloidogyne* sp.		*R. incultus*		*X. mampara f. minor*
	Criconemoides sp.	*Nanidorus*	*N. minor*		*R. karooensis*		*X. maraisae*
	C. sphaerocephaloides		*N. renifer*		*Rotylenchus* sp.		*X. meridianum*
	C. sphaerocephalus		*Nanidorus* sp.		*R. unisexus*		*X. mluci*
	C. xenopax	*Ogma*	*Ogma sp.*		*R. usitatus*		*X. ornativulvatum*
Discocriconemella	*Discocriconemella sp.*	*Paralongidorus*	*P. deborae*	*Scutellonema*	*S. africanum*		*X. ornatizulu*
Ditylenchus	*Ditylenchus africanus*		*P. hooperi*		*S. bizanae*		*X.paritaliae*
Dorylaimellus	*Dorylaimellus* sp.		*Paralongidorus* sp.		*S. brachyurus*		*X. simplex*
Geocenamus	*Geocenamus* sp.	*Paratrichodorus*	*P. lobatus*		*S. dreyeri*		*Xiphinema* sp.
	G. brevidens		*P. porosus*		*S. magniphasma*		*X. swartae*
Helicotylenchus	*H. digonicus*		*Paratrichodorus* sp.		*S. sorghi*		*X. vanderlindei*
	H. dihystera	*Paratrophurus*	*Paratrophurus sp*		*Scutellonema* sp.		*X. variabile*
	H. egptiensis	*Paratylenchus*	*P. obtusicaudatus*		*S. transvaalense*		*X. vitis*
	H. martini		*Paratylenchus* sp.		*S. truncatum*		*X. xenovariabile*
	H. microcephalus	*Pratylenchus*	*P. brachyurus*		*S. unum*		*X. zulu*
	H. minzi		*P. crenatus*				
	H. multicinctus		*P. delattrei*	*Subanguina*	*Subanguina* sp.		

Table 1.4 Continues

Genus	Species	Genus	Species	Genus	Species	Genus	Species
Helicotylenchus	*H. paraplatyurus* *H. pseudorobustus* *Helicotylenchus* sp. *H. vulgaris*	*Pratylenchus*	*P. fallax* *P. neglectus* *P. penetrans* *P. pratensis* *P. scribneri* *Pratylenchus* sp. *P. teres* *P. thornei* *P. vulnus* *P. zeae*	*Telotylenchus* *Telotylenchus*	*T. avaricus* *T. ventralis*		
Hemicriconemoides	*H. brachyurus* *H. strictthecatus*			*Trophotylenchulus*	*Trophotylenchulus* sp.		
Hemicycliophora .	*H. labiata* *H. lutosa* *Hemicycliophora* sp. *H. typica*			*Tylenchorhynchus*	*T. brevilineatus* *T. dewaeli* *T. goffarti* *T. mashhoidi* *Tylenchorhynchus* sp.		
Heterodera	*Heterodera sp.*	*Quinisulcius*	*Q. capitatus*	*Tylenchulus*	*Tylenchulus sp.*		
Histotylenchus	*Histotylenchus* sp.						

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

Three *Meloidogyne* spp. are reported from maize in South Africa, the two most common species reported are *M. incognita* and *M. javanica* (Mc Donald *et al*., 2017). *Meloidogyne arenaria* (Neal, 1889) Chitwood, 1949 also occur in local maize fields, but to a lesser extent than the aforementioned species. These species all have a wide host range (Moens *et al.,* 2009), which contributes to their high pest status and makes them difficult to control. Locally the known host range for these species is also extensive (Kleynhans, 1991; Kleynhans *et al*., 1996)

1.2.2.3.1.1 Life cycle

Root-knot nematodes are sedentary, endoparasitic. The infective, second-stage juveniles (J2) hatch from eggs when optimal environmental conditions prevailand move into the soil. The J2 then enter the epidermal layer of a root system of a suitable host plant and migrate to its central vascular system. In this area, they start to feed and grow into adult, pear-shaped, sedentary females or motile, vermiform males (Karssen *et al.*, 2013). A J2 penetrates and enters the roots of a host plant using a stomatostylet and feed on the cell contents (Fig. 1.7). The cells are injected with enzymes present in the saliva of the nematode that is transported through the orifice of the hollow stylet, which cause the plant cells to expand and form a feeding site (also called giant cells) which produces nutrients directly to the feeding nematode (Mitkowski & Abawi, 2003). Once this happens, the vermiform nematode becomes swollen and sedentary. Subsequently it molts and develops into a third- (J3) and fourth-stage juvenile (J4), which do not contain stylets and cannot feed. The female developes from the latter stage and is swollen and sedentary. She now has a feeding tube and proceeds to feed on the giant cells. The males are vermiform and although they do have stylets, they do not feed. The female root-knot nematode can produce hundreds to thousands of eggs in her lifetime (Karssen *et al*., 2013). When she reaches the stage where egg-production should start, the vulva is already positioned to release the eggs on the outside surface of the root. She excretes a protective gelatine matrix into which the eggs are released. Infectious J2 will then hatch from these eggs and from there they will moves through the soil to find the root of a host to penetrate and develop further to complete the life cycle as summarised above. The life cycle of most thermophilic *Meloidogyne* spp. can range between 20 to 30 days at temperatures of 25 to 30ºC (Greco & Di Vito, 2009; Shurtleff & Averre, 2000).

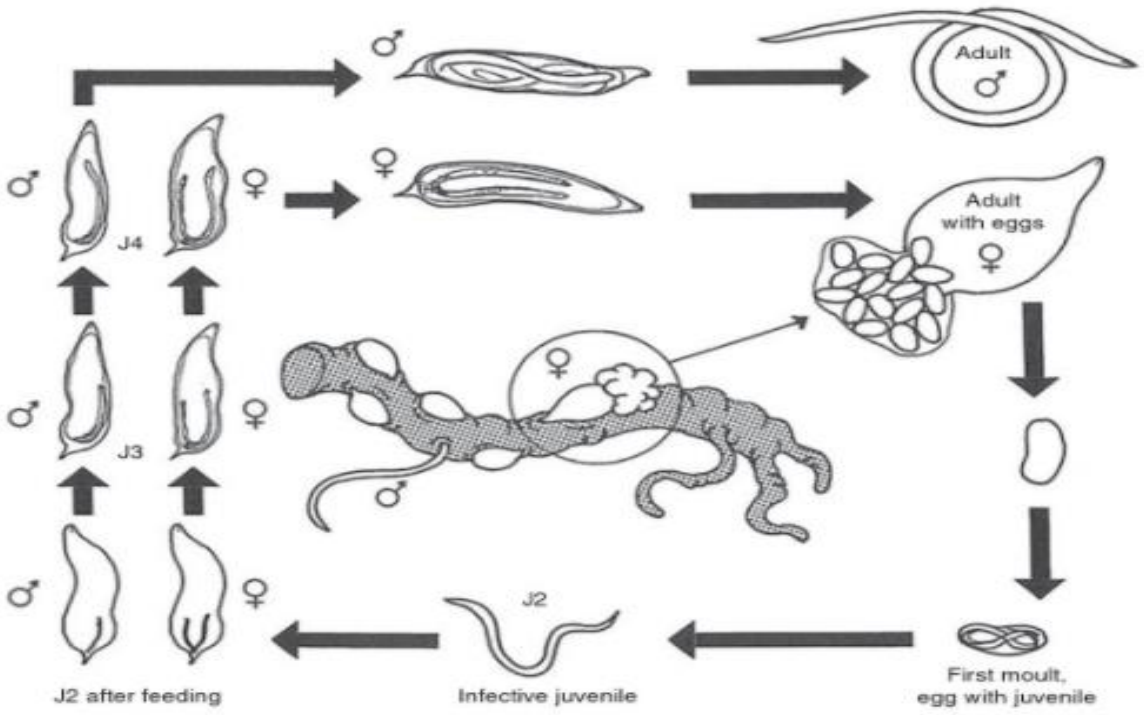


Figure 1.7 The life cycle of root-knot nematodes within the roots of a host plant (Moens *et al*., 2009).

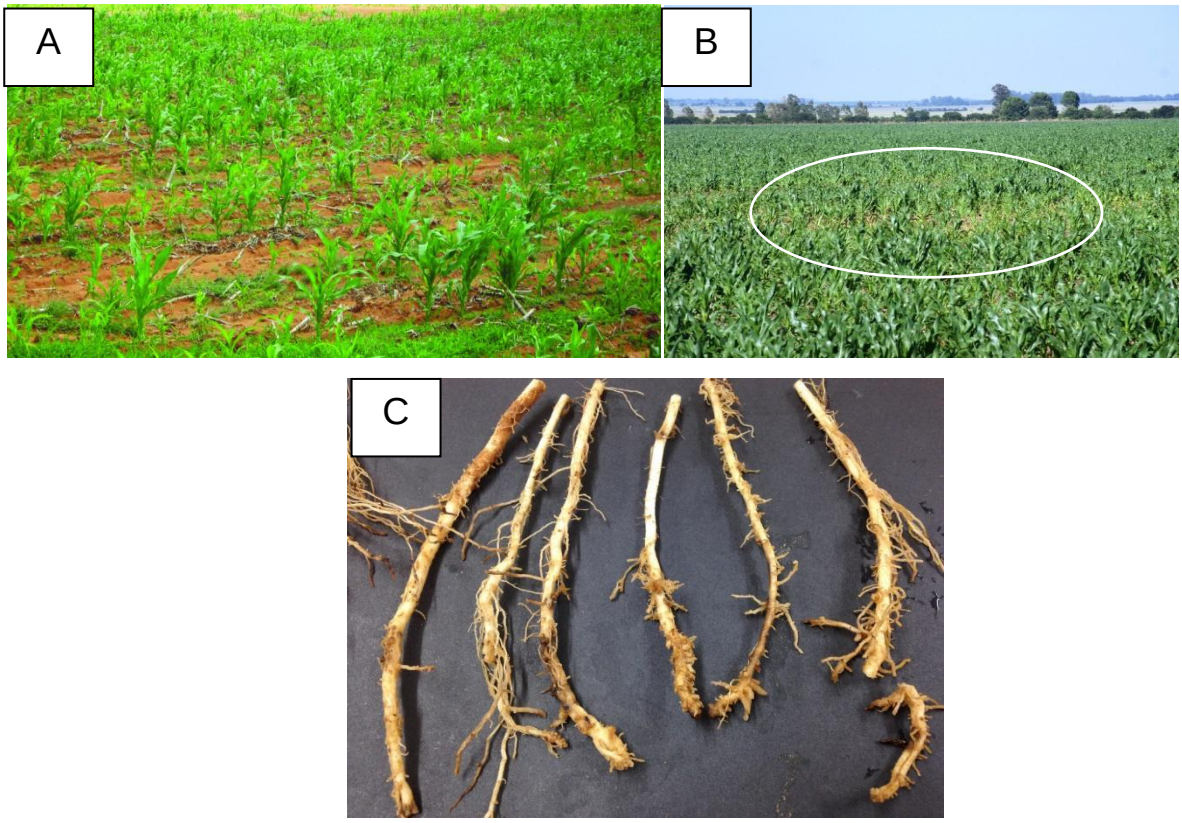
1.2.2.3.1.2 Reproduction strategies

Root-knot nematodes have two reproduction strategies namely sexual (amphimixtic) and asexual (parthenogenetic) reproduction (Chitwood & Perry, 2009). For amphimixtics, the presence of males is obligatory. Copulation between males and females occur and sperm are deposited in the spermatheca.. Conversely, parthenogenetic reproduction processes occur in the absence of males (Chitwood & Perry, 2009) where the eggs divide by means of meiosis for further development. This type of reproduction is a major contributor to success of some species as pathogens (Chitwood & Perry, 2009).

1.2.2.3.1.3 Symptoms and damage

Above-ground symptoms caused by plant-parasitic nematodes are usually visible as uneven patches of poor-growing plants in a maize field (Fig. 1.8 A and B). Such nematode infected plants may be stunted, have chlorotic leaves and/or in general do not appear healthy' (Fig. 1.8 A and B) when compared to their uninfected counterparts (Karssen *et al.*, 2013). Below-ground symptoms of nematode-infected maize roots may differ according o thepopulation densities. For example, seedlings that grow in patches heavily infected with root-knot nematodes may show root galling (Fig. 1.8 C). However, in older plants growing in these

patches, galls may not necessarily be visible. Other below-ground symptoms may include stunted roots with swollen tips (growth points) (Fig. 1.8 C) or roots with necrotic tissue such as those infected by lesion nematodes (Fig. 1.10).



Figures 1.8 Aboveground (A and B) and root (C) damage symptoms of *Meloidogyne* spp. parasitizing maize in South Africa. (Courtesy: Driekie Fourie and Suria Bekker, NWU (B and C) and Dr Chris Schmidt (A))

1.2.2.3.1.4 Interaction with other soil-borne organisms

There are many species of bacteria, fungi and viruses occupying the same ecological niche where nematodes are found, making a secondary infection by other pathogens more likely and add to the severity of disease to a host plant (Sankari Meena *et al.*, 2016). In most cases these disease complexes involve members of *Meloidogyne* spp., where the pathogens enter at the wound sites caused by the nematode entering and migrating through the root (Back *et al.* 2002). Infection of both fungi (e.g. *Fusarium* spp.) and nematodes can disrupt the ability of the host to take up water and nutrients, which can cause lower plant function and lead to greater yield losses (Sankari Meena *et al.*, 2016). Examples of fungi that thrive in host plants already infected by *Meloidogyne* spp. are species belonging to the

genera *Fusarium* Link, 1809; *Phytophthora* De Bary, 1875; *Verticillium* Nees, 1913 and *Rhizoctonia* De Candolle, 1815. (Taylor & Sasser, 1978). Examples of species of bacteria such as *Pseudomonas* Migula, 1894, *Agrobacterium* Conn, 1942 and *Corynebacterium* Lehmann & Neumann, 1896 have also been reported (Taylor & Sasser, 1978).

1.2.2.3.2 Lesion nematodes (*Pratylenchus* spp.)

Root-lesion nematodes are regarded as the second-most economic important plant-parasitic nematode genus worldwide (Jones *et al*., 2013). Individuals of this genus are widely distributed throughout temperate and tropical environments and they parasitize a wide variety of plant hosts. Lesion nematodes are migratory endoparasites that cause necrosis of root surface tissue and the cortex of plants because of feeding with a stomato stylet as they migrate intracellularly through the root (Davis & MacGuidwin, 2000). Locally, *Pratylenchus brachyurus* (Godfrey, 1929) Filipjev & Schuurmans Stekhoven, 1941 has been listed as the predominant lesion nematode species associated with local maize crops, followed by *Pratylenchus zeae* Graham, 1951 and other *Pratylenchus* spp. such as *P. crenatus* Loof, 1960; *P. delattrei* Luc, 1958; *P. fallax* Seinhorst, 1968; *P. neglectus* (Rensch, 1924) Filipjev & Schuurmans Stekhoven, 1941, *P. penetrans* (Cobb, 1917) Filipjev & Schuurmans Stekhoven, 1941, *P. pratensis* (de Man, 1880) Filipjev, 1936; *P. scribneri* Steiner, 1943; *P. teres* Khan & Singh, 1975; *P, thornei* Sher & Allen, 1953 and *P. vulnus* Allen & Jensen, 1951 (De Waele and Jordaan,1988; SAPPNS[1]).

1.2.2.3.2.1 Life cycle

The life cycle of lesion nematodes can range between four to eight weeks, depending on the environmental conditions and the presence of suitable plant hosts. Similar to root-knot nematodes and other nematode genera, the life cycle of lesion nematodes includes an egg, four juvenile stages and the mature males and females. They are migratory endoparasitesthat enter the root and move through the root system while feeding. All life stages of lesion nematodes are infective (Fig 1.9) (Jones *et al.* 2013).

[1]Dr Mariette Marais of the Nematology Unit, Biosystematics Division, Agricultural Research Council – Plant Health and Protection is thanked for the use of data from the South African Plant-Parasitic Nematode Survey (SAPPNS) database; E-mail: maraism@arc.agric.za

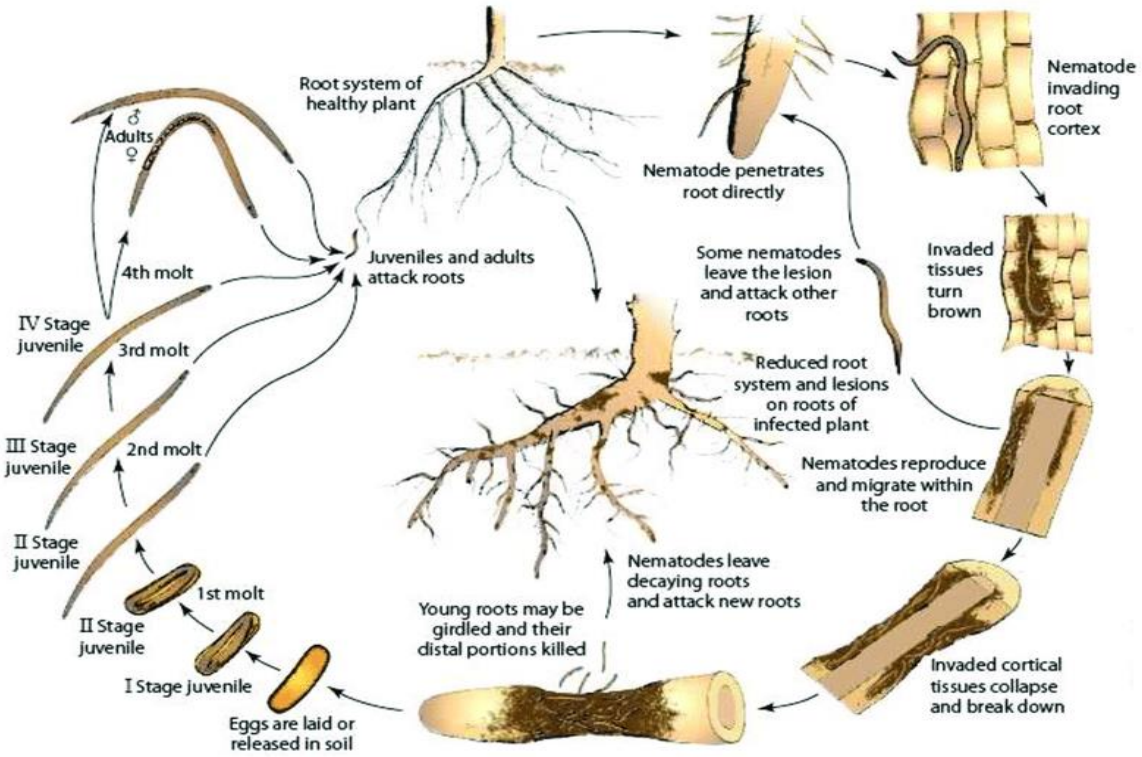


Figure 1.9 The life cycle of lesion nematodes (Mokrini *et al*., 2016).

1.2.2.3.2.2 Reproduction strategies

Lesion nematodes usually reproduce through amphimixis where male and female individuals are present. However, in the absence of males, females are able to reproduce through parthenogenesis (Davis & MacGuidwin, 2000).

1.2.2.3.2.3 Symptoms

Above-ground symptoms caused by lesion nematodes are similar to those caused by root-knot nematodes (see 1.2.2.3.1.3). Below ground symptoms i are, however, visible as brown/black lesions on maize roots, girlded young roots and pruned lateral roots (Fig. 1.10). Bellow ground symptoms may also include girdled young roots and pruned lateral roots. Such symptoms are, however, difficult to distinguish from symptoms caused by other pests and/or diseases that concurrently infest maize plants.

Figure 1.10 A maize root infected with lesion nematodes, resulting in brownish to blackish necrotic tissue (Courtesy: Danny Coyne, IITA).

1.2.2.3.2.4 Interactions with other soil-borne organisms

Interactions with other soil borne pests generally occur where fungi and bacteria use the entry wounds on the roots caused by lesion nematodes and this way cause further damage to the plant host (Back *et al.* 2002). *Pratylenchus* spp. commonly interact with the wilt fungii *Fusarium* and *Verticillium* and with root-rot pathogens *Pythium* Pringsheim, 1858, *Rhizoctonia* and *Phytophthora* (Back *et al.* 2002).

1.2.2.4 Identification of nematodes

The accurate identification of nematodes in general and *Meloidogyne spp.* specifically are fundamental for management and control of the specific species present in a maize field. Maize cultivars varies in susceptibility to the different root-knot nematode species and host plant resistance is species- or cultivar specific (Garcia & Sanchez-Puerta, 2012). Unfortunately, the accurate identification of *Meloidogyne* species have become challenging to even the most experienced nematologists. This is due to factors such as different life stages present in a field, wide host ranges of the nematodes, the complexity of different nematode species, polyploidy, sexual dimorphism and the presence of more than one *Meloidogyne* spp. in one field (Blok & Powers, 2009). The presence of more than one *Meloidogyne* spp. in one field further complicates accurate species identification of becasue of intraspecies and intrapopulation variances (Hunt & Handoo, 2009). According to Blok and Powers (2009), half of the *Meloidogyne* species have only been described recently and the possibility of finding a new one is, therefore, highly likely to occur.

Different life stages can be used for identification (juveniles, adult females and adult males) which can be extracted from either root or soil samples, depending on the type of research conducted (Blok & Powers, 2009). For example, bioassays involving nematicide testing or germplasm screening in which inoculum are used will have different requirements when sampled compared to that sampled for surveys or diversity studies (Blok & Powers, 2009).

1.2.2.4.1 Morphological and morphometrical approaches

Up to the early 2000s, morphological and morphometrical identification diagnostics was the most often-used, practical and cost-effective methods for the identification of *Meloidogyne* spp. (Jepson, 1987; Blok & Powers, 2009; Moens *et al.*, 2009; Garcia & Sanchez-Puerta, 2012). Morphological identification entails the study of perineal patterns, the head, stylet and oesophageal structures of females, the head region and stylet morphology of males and the characteristics of second stage juveniles (J2) (Eisenbach *et al.*, 1981; Hunt & Handoo, 2009; Onkendi *et al*., 2014). Morphometrics characters entails the measurement of different characteristics of J2, females and males such as body length, stylet length tail length, the distance from the head to the excretory pore and the position of the dorsal pharyngeal gland orifice (Hunt & Handoo, 2009; Jepson, 1987).

Classical identification can prove challenging, since a species such as *M. enterolobii* Yang & Eisenback, 1983, which is know seen one of the emerging root-knot nematode species, has morphometrical characteristics that overlap with that of other species such as *M. incognita*. These overlapping characteristics probably caused that *M. enterolobii* have been misidentified as M. incognita in the past (Blok & Powers, 2009, Hunt & Handoo, 2009). Recently, the use of deoxyribonucleic acid-(DNA) based methods in combination with morphological and morphometrical methods became more common and more efficient for the description and identification of *Meloidogyne* spp. because each method contributes its own advantages in the identification of a species (Onkendi *et al.*, 2014).

1.2.2.4.2 Biochemical and molecular approaches

Isozymes and antibodies are also used in *Meloidogyne* diagnostics (Blok and Powers, 2009). The use of these biochemical procedures added valuable information towards the accurate identification of especially *Meloidogyne* spp. through the examination of the isozymes phenotypes and antibodies (Blok & Powers, 2009).

1.2.2.4.2.1 Isozymes

In 1985, Esbenshade and Triantaphyllou described the use of enzyme phenotypes for the accurate identification of *Meloidogyne* spp.These authors used a thin-slab technique for a polyacrylamide gel electrophoresis that is specifically adapted to compare the enzyme profile of 20 to 25 different *Meloidogyne* females on the same gel (Esbenshade & Triantaphyllou, 1985). Esterase phenotypes proved to be an effective method used to distinguish between the major species of *Meloidogyne* (*M. incognita, M. javanica, M. arenaria* (Neal, 1889) Chitwood, 1949 *and M. hapla* Chitwood, 1949*.* This also applies for some of the less common species such as *M. chitwoodi* Golden, O'Banon, Santo & Finley, 1980 and *M. naasi* Franklin, 1965 (Esbenshade & Triantaphyllou, 1990; Blok and Powers, 2009).

A drawback of this method is that only young, adult females can be used for identification because the specific gene that is identified through isozyme phenotyping is only expressed in this life stage (Perry *et al.*, 2007). Another difficulty is that the determination of the band sizes of the different species requires the use of more than one enzyme to separate them (Esbenshade & Triantaphyllou, 1990). Despite these disadvantages, this method can be applied to verify and supplement other approaches in identifying *Meloidogyne* spp. (Blok & Powers, 2009).

1.2.2.4.2.2 Antibodies

The quality and quantity of DNA is crucial for the identification of a nematode species. A method was developed to enrich the extraction of the nematodes using an antibody-based capturing system because of the microscopic size of plant-parasitic nematodes and their irregular dispersal in the fields (Chen *et al.*, 2001). This method has an accuracy of up to 80 % and is applied to identify a single species from mixed spiecies populations using magnetic beads coated with a secondary antibody that recognises the surface of the target nematode species (Chen *et al.*, 2001, Blok & Powers, 2009).

1.2.2.4.3 Molecular diagnostic methods

An early example of the use of DNA-based methods, more specifically restriction fragment length polymorphisms (RFLP's), were published by Curran et al. (1985). The authors discovered that the restriction of endo-nuclease digestion of genomic DNA generated DNA fragments that had a unique size. These fragments of DNA can be visualised through agarose gel electrophoresis where a distinct band represented a specific species within the genera *Trichinella* Railliet, 1895*, Caenorhabditis* Osche, 1952*, Romanomermis* Nickle, 1972*, Steinernema* Filipjev, 1934 and *Meloidogyne* (Curran *et al.*, 1985)*.* After the discovery of the

polymerase chain reaction (PCR) technique, molecular diagnostics of nematodes gained field considerably (Nega, 2014). Several PCR-based methods that are implemented currently are discussed in the paragraphs that follow.

1.2.2.4.3.1 Extraction of DNA before molecular analysis

Before commencement of PCR, DNA must be extracted from individuals. This can be done for multiple or single species occuring in roots or from soil samples (Blok & Powers, 2009). The DNA of single, J2 individuals can be extracted by the physical crushing of the nematode using a pipette tip followed by adding sodium hydroxide (NaOH) or proteinase K and worm lysis buffer (Blok & Powers, 2009, Holterman *et al.,* 2006). There are also many commercially DNA extraction kits for single use available. After the DNA is extracted, the samples are ready for identification.

1.2.2.4.3.2 Restriction Fragment Length Polymorphisms (RFLPs)

This method involved the extraction and purification of genomic DNA, followed by the restriction digestion and visualization of banding patterns and gel electrophoresis (Blok & Powers, 2009). The highly repeated regions of the DNA banding patterns show the different samples that could be distinguished to species level (Curran et al., 1985). Unfortunately, it required a large amount of DNA, which meant that the isolates had to be cultured before the Restriction Fragment Length Polymorphisms (RFLP) process could continue (Blok & Powers, 2009). Despite these drawbacks, the method is still useful because it is not dependant upon a specific life stage or the inclusion of a complete genome (Blok & Powers, 2009). However, the lack of sensitivity and the complexity of this method can be be problematic if DNA from multiple *Meloidogyne* spp. are present. This issue led ultimately to the use of PCR through hybridization-based approaches of the RFLP method to identify *Meloidogyne* spp. (Blok & Powers, 2009).

1.2.2.4.3.3 Satellite DNA Probes

This method involves the detection and hybridization of satellite DNAs (satDNAs) in squashed nematode tissue with the use of a satellite probe. These probes target the heterochromatin, centromeric and telomeric regions of chromosomes (70-2000 bp in length) (Blok & Powers, 2009). It requires little molecular equipment or expertise and is usually applied when a large number of samples are analysed for species identification (Blok & Powers, 2009). SatDNA's have different signature sequences and can vary in their copy number, length and polymorphic regions for different *Meloidogyne* spp. (Blok & Powers, 2009).

1.2.2.4.3.4 Microarrays

The use of microarrays represents a new method where identification of species is conducted by means of oligonucleotide spotted arrays (Blok & Powers, 2009). This method was first reported to identify *Meloidogyne* spp. in the early 2000s (Francois *et al.*, 2006), but has not yet been widely implemented because it needs futher research for optimization. This approach can, however, be useful because it has the potential to analize a high quantity of samples simultaniously (Blok and Powers, 2009).

1.2.2.4.3.5 Real-time PCR

Real-time PCR is a high-cost identification method, using specialized reagents and equipment and is a fast and reliable method with increased sensitivity compared to that of conventional PCR (Blok & Powers, 2009). It can detect multiple species simultaneously with no post PCR procedures that need to be conducted (Blok & Powers, 2009). *Meloidogyne* spp. identification has recently been enhanced significantly using quantitative PCR assays (qPCR) (Blok & Powers, 2009, Onkendi *et al*. 2014).

1.2.2.4.3.6 Random Amplified Polymorphic DNA (PAPD)

With the use of Random Amplified Polymorphic DNA (RAPD's), annealing temperatures are lowered during the amplification cycle. This process allows the short RAPD primer (8 to 10 nucleotides long) to anneal at random in the genome (Cenis, 1993). By doing so, the synthesis of a highly polymorphic amplification DNA product is achieved, distinguishing other diverse organisms such as bacteria, fungi and plant cultivars (Cenis, 1993). The RAPD-PCR technique proved to be useful for diagnostic purposes and can be implemented to identify multiple and mixed *Meloidogyne* spp. populations within a few hours using only picogram amounts of DNA (Cenis, 1993).

1.2.2.4.3.7 Ribosomal DNA Polymerase Chain Reaction

The PCR approach enables nematologists to make numerous identical copies from a single or a few nematode DNA molecules (Curran et al., 1985). In 1994, Ibrahim and co-authors used PCR to identify 12 species of *Aphelenchoides* Fischer, 1894 and *Ditylenchus angstus* (Buther, 1913) Filipjev, 1936 populations (Ibrahim *et al*., 1994). In this technique various sets of PCR primers were used to amplify the ribosomal DNA (rDNA) of the specific nematode species (Blok & Powers, 2009). In effect, PCR primers have been used for phylogenetic and diagnostic studies and are based on sequences in the 18S, 28S and 5.8S ribosomal genes and also the internal transcribed spacer (ITS), external transcribed spacer (ETS) and intergenic spacer (IGS) regions (Fig 1.5) (Blok & Powers, 2009).

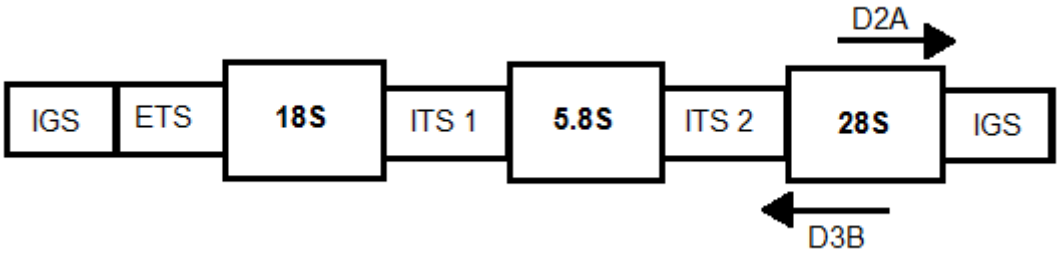


Figure 1.5 Image to indicate the ITS1-5.8S-ITS2 ribosomal DNA segment (Douda *et al.*, 2013)

Primers especially designed to amplify the ITS region have been widely used to identify members of the *Meloidogyne* spp. (Blok & Powers, 2009). There are reports of some limitations experienced with ITS sequencing of *M. incognita, M. javanica* and *M. arenaria*, is that polymorphisms that these species showed some gene lineages and can therefore be misidentified through the standard ITS sequencing (Blok & Powers, 2009). To eliminate this error, specific sequenced characterized amplified region (SCAR) primers were developed.

1.2.2.4.3.8 Sequenced Characterized Amplified Region PCR

The Sequenced Characterized Amplified Region (SCAR) primers were developed from RAPD to amplify diagnostic repetitive regions that identify several *Meloidogyne* spp. (Zijlstra *et al*., 2000, Blok & Powers, 2009). The diagnostic repetitive regions were identified through analyzing isolates of seven *Meloidogyne* spp. that had short RAPD primers (8 to 10 nucleotides) (Blk & Powers, 2009). These primers proved to be less sensitive than the RAPDs and less likely to produce banding patterns for contaminants (e.g. microbial contaminants) (Zijlstra *et al*., 2000). SCAR-PCR is a quicker and easier way to identify *Meloidogyne* spp.. However, in this ever-changing world, information on nematode DNA sequencing constantly needs to be updated to design new efficient primers, which makes SCAR-PCR an expensive process (Cenis, 1993). The current study used this diagnostic approach to identify *Meloidogyne* spp. and is further discussed in Chapter 3.

1.2.2.4.3.9 D2/D3

The D2/D3 expansion segments forms part of the ribosomal DNA (rDNA) unit in the 28S-LSU region (Douda *et al*., 2013). The rDNA unit has become more popular to use for phylogenetic diagnosis in the past decade because of its highly variable and conserved regions among different organisms (Telente *et al.*, 2004). This author also proved,where he compared the D2/D3 region of eight different *Meloidogyne* spp., that the use of this rDNA region is more suitable to distinguish separate species groups rather than for species specific diagnostics (Telente *et al.*, 2004, Douda *et al*., 2010).

1.2.2.4.3.10 Mitochondrial DNA PCR

The use of Mitochondrial DNA (mtDNA) as a baseline to distinguish between different *Meloidogyne* spp., have become more popular over the last decade (Onkendi *et al*., 2014). Even though mtDNA evolves rapidly in nematodes, the variation among individuals of the same species proved to be a fraction of a percent to 2 % (Blouin, 2002). The regions mostly sequenced for diagnostic purposes include the cytochrome oxidase subunits and the NADH subunits (Fig 1.6) representing loci from mitochondrial protein groups that show both high (cytochromes) and low (NADH dehydrogenase group) conservation (Blouin, 2002).

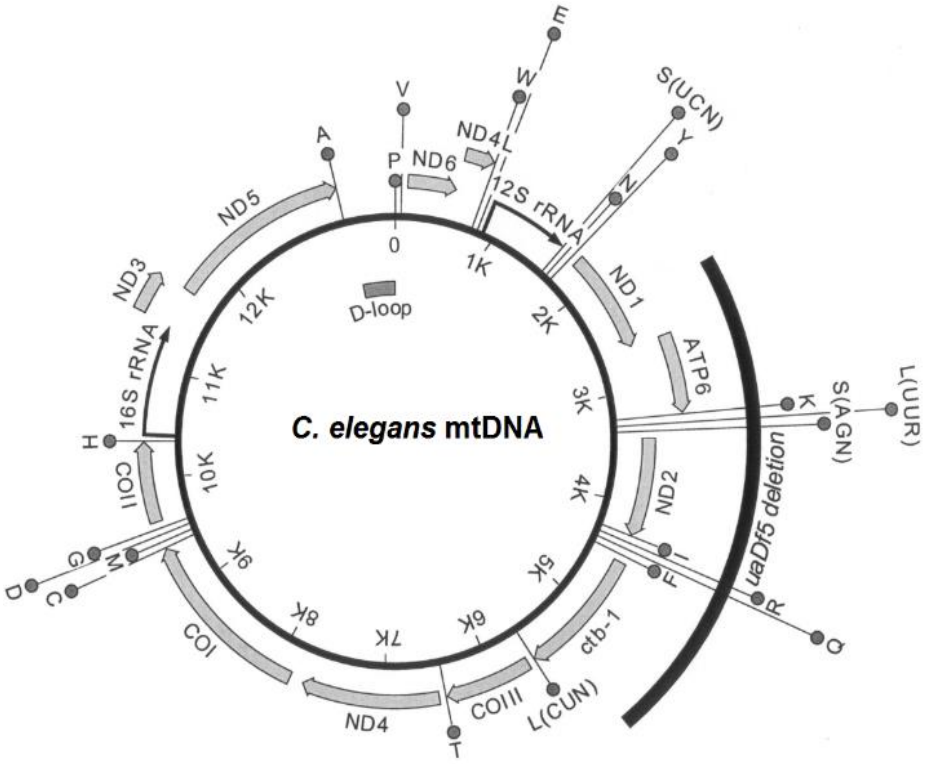


Figure 1.6 The gene-map of *C. elegans* mtDNA (Lemire, 2005)

The cytochrome oxidase subunits I and II (COI and COII) genes are located in the 16S rRNA region of mitochondrial DNA (Figure 1.6). These regions are sequenced to identify multiple *Meloidogyne* spp. with different sized amplified products. For example, the COII products were derived from the 3' portion of the COII gene and the 5' portion of the 16S rRNA region (Blok & Powers, 2009, Powers *et al.*, 1993). According to Blouin (2002) the use of the NADH dehydrogenase group appears to be more suited for sequencing than the COI and COll genes because of the strong amino acid conservation of the latter that limit most of the useful variations to silent sites. The NADH dehydrogenase 5' (NADH5) gene is also situated on the 16S rRNA genome (Figure 1.6).

Unfortunately, most of the rDNA and mtDNA-based identification methods tend to have shortcomings. It is therefore crucial for all diagnostic purposes to test for more than one target gene, whether these target genes are in the same gene or in another (Onkendi & Moleleki, 2013). Furthermore, complementing molecular analyses with morphological and morphometrical diagnostic approaches is still preferred to enhance accurate results in the identification of *Meloidogyne* spp. (Onkendi *et al.*, 2014). The correct identification of a nematode pest is crucial to be able to implement the right control strategies for that specific species and to contribute to our knowledge of their dispersal and pathogenicity throughout South Africa.

1.2.2.5 Management of *Meloidogyne* spp.

Integrated pest and disease management (IPM) are the most successful control strategy to manage and control agricultural pests throughout the world (Ehler, 2006). The goal of plant-parasitic nematode management is to decrease their population densities to where the damage caused will not have a substantial impact on the total yield production (Moens e*t al.,* 2009). Total eradication of nematode pests is, however, impossible (Moens e*t al.,* 2009). Although various strategies are implemented worldwide to reduce nematode pest populations, most producers depend on chemical control to address nematode problems in maize plantings (Mc Donald & Nicol, 2005). The progressive withdrawal of nematicides, however, increased the pressure on scientists to investigate and develop alternative management strategies to combat nematodes (Moens *et al.,* 2009). Other management strategies used to combat nematode pests include the use of resistant cultivars, cultural control (e.g. crop rotation with poor or non-hosts), biological control, trap cropping and fallowing (Coyne *et al*., 2009). For the purpose of this dissertation only those strategies most commonly used to control plant-parasitic nematode populations in maize in South Africa will be discussed.

1.2.2.5.1 Chemical control

The use of nematicides started in the early 1950s (Moens *et al*., 2009). During the 1970s, the danger that some chemical nematicides posed to animals, humans and the environment was acknowledged. Some nematicides became restricted and were eventually banned completely (Moens *et al*., 2009), including aldicarb (Senwes, 2012) and endosulfan (Verdoorn, 2012). Nematicides that are still registered for use in maize fields in South Africa include fumigants, granular and liquid formulations (Agri-Intel, 2017). The active-ingredient

groups to which such Class I, red-band products belong include carbofuran, carbosulfan, furfural and terbufos.

Nematicides registered on maize are not species specific and will control most nematode pests associated with the crop. All the synthetically-derived nematicides registered for use on maize in South Africa (Agri-Intel, 2017) also have insecticidal properties (Van Zyl, 2013), which are to the benefit of the producer. Where high infestation levels of nematode pests, root-knot nematodes in particular, prevail in irrigated maize, the application of a nematicide is recommended as a general rule. The use of granular nematicides often result in yield increases of >2 metric tonnes per hectare, illustrating substantial net economic return for producers depending on the maize price (Riekert, 1998).

The use of nematicides is still the most common nematode control strategy used by local maize producers (Mc Donald *et al*., 2017) and the use thereof, especially by local commercial maize producers in both rain-fed and irrigated fields, escalated over the last two decades. Little attention has been paid to the economic feasibility of such nematicides, however, even though the economic viability of a nematicide treatment is crucial for South African maize producers. This especially applies to those producing maize under rain-fed conditions, which in itself poses a major and inherent risk factor (Mc Donald *et al*., 2017). During the 2012/13 growing season, more than 85 % of both white and yellow maize were cultivated under dry-land conditions (DAFF, 2014). The use of a nematicide will only be feasible if the financial gain of a maize crop exceed the cost of a nematicide application. Therefore, synthetic nematicides are generally too expensive for developing producers (Mitkowski & Albawi, 2003) and for use under rain fed conditions over a large area (Riekert, 1996).

1.2.2.5.2 Cultural control

Cultural control is regarded as one of the most environmentally sustainable approaches to manage plant-parasitic nematodes because the different methods (e.g. crop rotation, cover cropping, soil tillage and fallow) categorised under this strategy does not involve the use of toxic chemicals and thus pose no danger to animals, humans and the environment. In some cases, however, it can be costly because of specialized equipment that may be needed to deal with the different crops planted (Mitkowski & Abawi, 2003). Also very important is that knowledge about the initial population densities (Pi) of the target nematode pest be known. However, for South Africa little data is available.

Very important for effective cultural control is that the initial population density (Pi) of the target nematode is known (Nyczepir & Thomas, 2009; Coyne *et al.,* 2009). Unfortunately, literature about the relationships between yield loss of maize and Pi of plant-parasitic nematodes, particularly *Meloidogyne*, is scarce and fragmented. Only a few studies have been published for *M*. *incognita* and *M javanica* (Di Vitto *et al*., 1980; Fourie *et al*., 2008). A strong non-linear relationships between Pi (0 to 20 000 eggs and J2 per seedling) and final population densities (Pf) of *M. incognita* and *M. javanica* were reported for resistant and susceptible maize genotypes in a South African study (Fourie *et al*., 2008). Yield losses for the susceptible cultivars at Pi level of 20 000 eggs and J2/seedling were 55 % and 72 % for *M. javanica* and *M. incognita*, respectively. From Italy, Di Vito *et al*. (1980) reported an inverse relationship between Pi and Pf population densities for a maize hybrid. In their study, the lowest Pi of 0.5 eggs of *M. incognita* per gram soil at planting resulted in a 458-times increase in Pf at harvest. Moreover, a strong non-linear relationship existed between Pi and maize yield, with a damage threshold level of 10 eggs per gram of soil being recorded. Ultimately, growth reduction of inoculated maize plants was 90 % compared to that for non-inoculated (control) plants.

Crop rotation is a common cultural strategy that aims to distance susceptible crops in space and time in a system from the target nematode species to reduce the nematode population to under the economic threshold (Coyne *et al*., 2009). The success of crop rotation as a strategy, however, depends on knowledge about identity of the target nematode species that occur in a specific crop field, population densities of such a pest and the host status of cultivars of the crops to be planted (Nyczepir & Thomas, 2009; Coyne *et al.,* 2009). Planting poor-host crops in rotation with maize can reduce the population densities of target nematode species and ultimately, lower yield losses. However, the presence of more than one plant-parasitic nematode species in a specific field is, in most cases, a reality. Therefore, finding a non- and/or poor host crop cultivar for multiple nematode species is a difficult task (Luc *et al.*, 2005). Another challenge faced in implementing effective crop rotation is the wide host ranges and quick population build-ups of economically-important plant-parasitic nematode genera such as *Meloidogyne* and *Pratylenchus* (Mitkowski & Abawi, 2003; Luc *et al.*, 2005). Summer crops usually rotated with maize locally include dry bean (*Phaseolus vulgaris* L.), groundnut (*Arachis hypogaea* L.), soybean (*Glycine max* L. Merr) and sunflower (*Helianthus annuus* L.). In winter, small cereal crops such as wheat (*Triticum aestivum* L.) are grown in some areas of the country. These crops are all known

hosts of root-knot nematodes (Kleynhans *et. al*., 1996; Onkendi *et al.*, 2014; Mc Donald *et al.*, 2017) and contribute to the increase of nematode populations in maize fields.

Another cultural control method implemented on a large scale in commercial maize production areas of South Africa in particular, is the use of soil tillage (Du Plessis, 2003). Cabanillas *et al*. (1999) recommended the combination of tillage and crop rotation as part of a nematode management strategy. In some studies, tillage had little effect on nematode densities, while in other studies nematode assemblages were greatly influenced by this method (Mc Donald and Nicol, 2005). The important influence of environmental factors in these systems and inter-relationships cannot be overestimated and should always be taken in consideration (Yeates and Hughes, 1990). Repeated tilling of the upper 30 cm soil layer at 30-day intervals during the hot and dry seasons between crops, can contribute significantly to reducing nematode pest densities through the desiccation of eggs and J2 (Sikora *et al*., 2005). This practice, however, is not always economical because of the possible impact of the loss of soil moisture after tilling on the current and successive crops.

Other cultural strategies such as the planting of cover crops (Luc *et al.*, 2005), crops used for amendment and bio-fumigation characteristics (i.e. Brassicaceae) (Ploeg, 2008) and antagonistic crops (*Tagetes patula* L.*, T. erecta* L. and *T. minuta* L.) (Wang *et al.,* 2007; Coyne *et al*., 2009) are also used mainly to assist in reducing nematode pest population levels in small fields such as those planted by small-holder famers (Coyne *et al*., 2009). However, these methods are often economically not feasible in the biggest part of local production areas because of the high irrigation costs involved and the limited availability of water in rain-fed areas (Chitwood, 2002).

1.2.2.5.3 Genetic host plant resistance

The use of host plant resistance is a popular and environmentally-friendly strategy to combat nematode, especially for producers on modern high-input production systems (Karssen *et al*., 2013). Resistant traits inhibit development and reproduction of the target nematode pest, which alleviates the adverse effects of these nematodes on crop yield and/or quality (Moens *et al*., 2009; Karssen *et al*., 2013). Identification of resistant genes in maize has been done in various countries such as South Africa (Ngobeni *et al*., 2010), the USA (Windham and Williams, 1994), Nigeria (Adegbite, 2011) and Uganda (Kagoda *et al*., 2011). Various cultivars resistant to South African populations of both *M. incognita* and *M. javanica* have been identified by Ngobeni *et al*. (2010) while other cultivars were identified as resistant to

one of these species but not to the other. On the other hand, DKC8010 and AFG4410, proved to be highly susceptible to both of these nematode species.

It should be taken in consideration, however, that maize and other crops are currently being screened for their host status to *Meloidogyne* spp. only. Since *Pratylenchus* spp. is also an economically important pest of maize, future studies should aim to also screen maize cultivars and those of crops used in rotation with maize against the *Pratylenchus* spp. that prevail in local maize production areas.

1.2.2.5.4 Alternative control options

The effects of other products on nematode pests associated with local maize crops including a seaweed product (De Waele et al. 1988) and various herbicides (De Waele & Jordaan, 1988), have been evaluated for their efficacy against *P. zeae*. None of the products were able to effectively reduce lesion nematode species, however.

Biological agents that include living organisms or antagonists such as predators and parasites of organisms that damage or kill their hosts (Moens *et al*., 2009), have also been investigated for their effect in reducing especially root-knot nematodes in maize (Mc Donald *et al*., 2017). Only two biologically based products are currently registered on maize in South Africa namely i) the seed treatment Avicta® 500FS (Van Zyl, 2013) with secondary metabolites of the soil-inhabiting bacterium *Streptomyces avermitilis* as the active substance and ii) Poncho®VOTiVo® with the active substance being secondary metabolites of the bacterium *Bacillus firmus* (Bayer, 2016). However, the harsh conditions in maize fields, especially those in rain-fed areas, decrease the affectivity of biological agents.

Avicta® 500FS contains abamectin as the active substance which represents macrolyctic lactones that are produced by *S. avermitilis.* This active substance are effective against a wide range of nematodes (Faske & Starr, 2006). This commercially available product is reported to effectively control *Meloidogyne* spp. in a wide range of crops, including maize (Van Zyl, 2013). Abamectin has a low water solubility and rapid degradation rate in soil, meaning that it is unlikely to contaminate soil water (Garabedian & Van Gundy, 1983). This product is hence considered environmentally more friendly and more cost-effective than Class I nematicides (Cabrera *et al*., 2009).

1.2.2.5.5 Preventative strategies

Preventing the introduction and spread of plant-parasitic nematodes (Moens *et al*., 2009; Karssen *et al*., 2013) in crop fields is the first important step to alleviate damage. Focusing on maize and other annual rotation crops in particular, the spread of root-knot nematode pests through farming implements to which soil containing the pest adheres(Moens *et al*., 2009; Karssen *et al*., 2013), needs to be prevented. Furthermore, the risk of spreading *Meloidogyne* spp. through infected plant parts (Moens *et al*., 2009; Karssen *et al*., 2013) such as potato tubers can also occur in production areas where these crops are included as a rotation crop.

In order to control and manage nematode pests effectively in maize-based cropping systems, an integrated management approach, such as the use of synthetic nematicides, seed treatments and host plant resistance, needs to be developed and applied. Soils can also be enriched by application of organic material or the use of cover crops to increase the diversity of soil-inhabiting biota.

1.3 Hypothesis and aims of this study

The hypotheses for this stydy is that root-knot nematodes will be the most abundant group that parasitices maize in South Africa and that an increase of initial Pi of the two predominant root-knot nematode species identified in the first hypothesis will differ in terms of final population densities (Pf).

The main aim of this study was to re-assess the abundance, diversity and occurrence of plant-parasitic nematodes in local maize fields, to evaluate the effect of the nematode pest associated with local maize crops and determine the relationships between Pi and Pf of *Meloidogyne* spp., and the effect of a registered nematicidal seed treatment for a susceptible maize cultivar.

The individual objectives of this study were to:
i) conduct a nematode survey in selected local maize production areas to assess the abundance, diversity and occurrence of plant-parasitic nematodes present.
ii) conduct molecular identification of the economical most important plant-parasitic nematode species, foreseen to be *Meloidogyne* spp., identified during the survey.

iii) evaluate the effect of different initial Pi levels of the predominant *Meloidogyne* spp. nematode pest species on a susceptible and resistant maize cultivar genotype, and
iv) determine the effect of a nematicidal seed treatment on the population development of *Meloidogyne* spp. for a susceptible maize cultivar.

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Chapter 2: A re-assessment of plant-parasitic nematodes infecting maize, with emphasis on *Meloidogyne*

2.1 Introduction

Maize (*Zea mays* L.) is one of the largest produced field crops in South Africa, with approximately 15 631 million metric tonnes harvested from 19 468 million hectares during 2016 (GrainSA, 2017). Since people in developing countries consume maize as grain, meal and/or greenmealies, the crop serves as a major staple food source. A substantial part of crop rests are also fed to livestock as hay or silage (DAFF, 2008).

The production of local maize crops is hampered by various diseases and pests of which plant-parasitic nematodes are inclusive (Mc Donald *et al*., 2017). According to the latter authors and the South African Plant Parasitic Nematode Survey's (SAPPNS[1]) data base, the most common nematode pests of maize are root-knot (*Meloidogyne* Goeldi, 1887) and lesion (*Pratylenchus* Filipjev, 1936) nematodes. These and other nematode genera have been associated with maize yield losses ranging between 12 to 60 % (Louw, 1982; Keetch, 1989; Riekert, 1996; Riekert & Henshaw, 1998). The commonly occurring and most abundant *Meloidogyne* spp. recorded to parasitise maize and rotation crops used in maize-based cropping systems in South Africa, are *Meloidogyne incognita* (Kofoid & White, 1919) Chitwood, 1949 and *Meloidogyne javanica* (Treub, 1885), Chitwood, 1949 (Keetch & Buckley, 1984; Kleynhans *et al*., 1996; Riekert, 1996; Riekert & Henshaw, 1998). A third species, *Meloidogyne arenaria* (Neal, 1889) Chitwood, 1949 has also been reported from local maize production areas, but to a lesser extend regarding its occurrence compared to *M. incognita* and *M. javanica* (Agenbag, 2016; SAPPNS[1]). The latter two root-knot nematode species have been recorded as generally occurring as either monoculture or mixed populations in local maize fields (Louw, 1982; De Waele & Jordaan, 1988; Kleynhans *et al*., 1996), while *M. arenaria* also occur with them in mixed populations (Agenbag, 2016).

Accurate identification of *Meloidogyne* spp. is becoming increasingly important to enable the design of effective nematode management strategies such as crop rotation and genetic host plant resistance (Ye *et al*., 2015). Identification of *Meloidogyne* spp. using morphological and morphometric techniques, however, poses a challenge due to large intraspecies variation, especially where experienced nematode taxonomists are not available. Molecular identification of *Meloidogyne* spp. gained momentum during the past two decades (Adam *et al*., 2007), is common and used routinely in many studies.

Deoxyribonucleic acid (DNA) based techniques are quick and generally more reliable than morphological identification (Adam *et al*., 2007). Although several molecular techniques are nowadays used to identify *Meloidogyne* spp., the sequence-characterised amplified region based polymerase chain reaction (SCAR-PCR) is a powerful, accurate and rapid technique (Zijlstra, 2000; Zijlstra *et al*., 2000; Blok and Powers, 2009; Subbotin *et al*., 2013). Another technique, namely NADH dehydrogenase and specifically the subunit 5 (Nad5) gene fragment, has also been used with success to reliably identify root-knot nematodes belonging to the *M. incognita* group (MIG) (Janssen *et al*., 2016). A major benefit of using these two techniques is that as little as one or a few individuals of any life stage of a particular *Meloidogyne* spp. can be used to characterise their DNA (Adam *et al*., 2007). Various researchers in South Africa have successfully identified *Meloidogyne* spp. local species with different molecular techniques (Fourie *et al*., 2001; Berry *et al*., 2008; Onkendi & Moleleki, 2013).Although it is preferable to use molecular techniques in combination with biochemical and/or morphological methods, it is not always possible especially without experiences morphological diagnosticians and where a lot of populations have to be identified.

Concerning *Pratylenchus*, the predominant species associated with local maize crops are *Pratylenchus zeae* Graham, 1951, followed by *P. brachyurus* (Godfrey, 1929) Filipjev & Schuurmans Stekhoven. Other *Pratylenchus* species include for example *P. crenatus* Loof, 1960, *P. delattrei* Luc, 1958, *P. neglectus* (Rensch, 1924) Filipjev & Schuurmans Stekhoven, 1941, *Pratylenchus penetrans* (Cobb, 1917) Filipjev & Schuurmans Stekhoven, 1941, *P. pratensis* (De Man, 1880) Filipjev, 1936 and *P. vulnus* Allen & Jensen, 1951 (De Waele and Jordaan, 1988; Mc Donald *et al*.,2017; SAPPNS [2]). Like root-knot nematodes, lesion nematodes also have a wide host range (Bridge & Starr, 2007) and parasitise roots/other below-ground parts of most crops used in rotation with local maize (Kleynhans *et al*., 1996; Jones *et al*., 2017; Mc Donald *et al*., 2017; Van den Berg *et al*., 2017). Therefore, population densities of this nematode genus can build up to high levels and pose a threat to local maize producers.

Except for *Meloidogyne* and *Pratylenchus* spp., a wide range of other plant-parasitic nematode genera have also been associated with local maize crops. Some of these include for example various genera of the family Criconematoidae Taylor, 1936, while the genera *Ditylenchus* Filipjev, 1936, *Helicotylenchus* Steiner, 1945, *Longidorus* Micoletzky, 1922,

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Nanidorus Siddiqi, 1974, *Paratrichodorus* Siddiqi, 1974, *Paralongidorus* Siddiqi, Hooper & Khan, 1963, *Quinisulcius* Siddiqi, 1971, *Rotylenchulus* Linford & Oliveira, 1940, *Rotylenchus* Filipjev, 1936, *Scutellonema* Andrássy, 1958, *Telotylenchus* Siddiqi, 1960,*Tylenchorhynchus* Cobb, 1913, and *Xiphinema* Loos, 1950 (Keetch & Buckley, 1984; Louw, 1982; De Waele & Jordaan, 1988; Kleynhans et al., 1996; SAPPNS[1]) have also been listed. Such nematode pests, however, generally occur sporadic and in relatively low population levels, and are thus not regarded as threats to producers.Nonetheless, it is important that researchers monitor their presence in soil and/or root samples of maize so that should their abundance or occurrence increase, strategies can be followed to combat them.

At present, synthetically-derived nematicides from only four active ingredient groups, *viz*. aldehydes, carbofuran, carbosulfan and terbufos, are available to be used for local maize crops (Agri-Intel, 2017). Two alternative, environmentally friendly seed treatments are also currently registered on maize in South Africa, namely Avicta® 500FS (contains secondary metabolites of the soil-inhabiting bacterium *Streptomyce savermitilis* as its active ingredient) (Van Zyl, 2013) and Poncho®VOTiVo® (contains *Bacillus firmus* as the active ingredient) (Bayer Cropscience, 2017). Due to i) the progressive withdrawal of toxic, Class I nematicides (Verdoorn, 2012), ii) the progressive increase in nematode pest population densities in local maize fields according to diagnostic and research results (Bekker *et al*., 2007) and iii), the previous formal nematode-maize survey being done nearly 30 years ago (De Waele and Jordaan,1988) the need existed to re-assess the status and identity of plant-parasitic nematodes (particularly that of the predominant *Meloidogyne* spp.) in South African maize production areas. Therefore, the aims of this study were to i) conduct an extensive nematode survey across selected maize production areas, including fields under both rain-fed and irrigation conditions, to obtain data on the abundance, diversity and population densities of predominant nematode pests and ii) identify the *Meloidogyne* spp. by means of molecular techniques.

2.2 Materials and methods:

2.2.1 Survey

Samples were taken from 78 maize fields (Figure 2.1) in the North West, Free State, Gauteng, Northern Cape, Limpopo and Mpumalanga provinces during the 2013/14 growing season. Since this study was funded by Syngenta South Africa, the areas sampled were selected by their personnel and included mostly sandy soils. For each field, 32 root and corresponding soil samples were obtained using a zig-zag sampling method (Kleynhans *et*

al., 1997; Marais *et al*., 2017). Sampling was hence done representatively across each field during the flowering of the maize plants since population densities of plant-parasitic nematodes peak during this growth stage (Riekert & Henshaw, 1998). A garden spade was used to remove the entire root system of maize plants from the soil after which the roots, with adhering soil, were put into labeled plastic bags. The samples were kept in cool bags and out of direct sunlight, transported to the Nematology Laboratory of the North-West University (Potchestroom) and stored in a cold room (4 – 6 °C) until nematode extractions could be done. This procedure was done usually within five to fourteen days after the samples were collected. The 32 samples were then combined to ultimately form eight sub-samples per field that were prepared for nematode analyses (including extractions, counts and identification) by using the combined sampling method (Kleynhans *et al*., 1997). Information about the 78 fields, e.g. location, crop history, nematicides used and soil characteristics were obtained from the respective producers and are listed in Table 2.1.

Rainfall and temperature data were partially obtained from the producers in whose fields the samples were taken and from the website Climate-data.org (2017). Cultivars planted by producers are listed in Annexure Table 1.19. Not all info could, however, could be obtained regarding the cultivar names grown on all fields. Those for which information could be gathered are included in Table 2.20 in the Annexure.

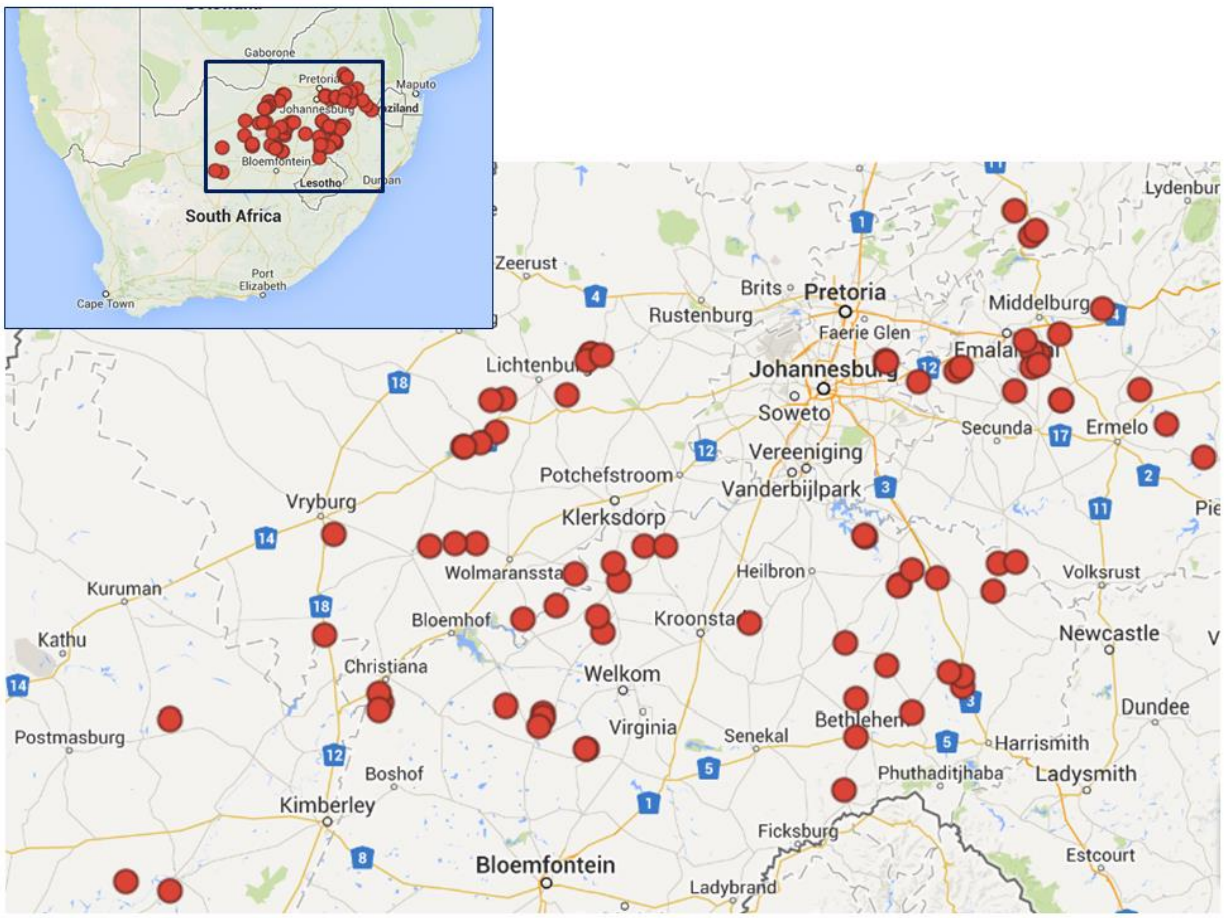


Figure 2.1 The location of 78 maize fields (each represented by a red circle) where root and soil samples were obtained in selected maize production areas of South Africa during the 2013/14 growing season for nematode analyses (During construction of the map, some fields overlapped and cannot be distinguished) (Photo: Google Maps, www.googlemaps.co.za).

Table 2.1 The 78 maize fields sampled for a nematode survey during the 2013/14 growing season, with information about the production practices used by the respective producers as well as data about selected soil characteristics.

Sample ID	Name of farm or producer	Town	Province	Rain fed (R) or Irrigation (I)	Crop history	Nematici-des used	Tillage practice	pH (H_2O)	Sand %	Silt %	Clay %	C %
1	Calby Farming	Christiana	North West	I	Potato, maize, grass	EDB	Conventional	7,38	92	1	7	0,16
2	C Forbes	Amsterdam	Mpumalanga	R	Maize	None	Conventional	5,72	61	15	24	1,99
3	G Mulder	Hendrina	Mpumalanga	R	Soybean, maize	None	Rip + disc	7,86	82	6	12	0,64
4	D Wensel	Vrede	Free State	R	Maize, soybean	None	Rip	5,53	58	22	20	1,08
5	Jappie VTV Bdy	Christiana	North West	I	Maize	None	Conventional	7,33	92	0	8	0,26
6	M Labouschagne	Wolmaransstad	North West	R	Maize	None	Conventional	6,80	92	0	8	0,30
7	W Easby	Christiana	North West	I	Potato, maize	None	Conventional	7,51	90	2	8	0,28
8	M Allan	Middelburg	Mpumalanga	R	Maize, soybean	None	Rip	6,31	79	5	16	0,67
9	M Erasmus	Hendrina	Mpumalanga	R	Maize, potato	None	Conventional	7,04	88	4	8	0,22
10	B Haasbroek	Schweizer-Reneke	North West	R	Maize	None	Conventional	6,50	87	3	10	0,35
11	B Rheeder	Makwassie	North West	R	Maize	Ortophos	Rip	6,61	88	3	9	0,27
12	D de Water	Bethal	Mpumalanga	R	Soybean, maize	None	Rip	6,27	87	5	8	0,37
13	K Marais	Stoffberg	Mpumalanga	R	Soybean, maize	None	No-till	7,50	46	14	40	1,43
14	J Breytenbach	Fouriesburg	Free State	R	Wheat, maize, sugarbean	None	Rip + disc	6,87	66	17	17	2,03
15	H Prinsloo	Kendal	Mpumalanga	I	Potato, oat, maize	None	Minimum tillage	6,23	77	6	17	0,67
16	J Randall	Lothair	Mpumalanga	R	Maize, soybean	None	Minimum tillage	6,18	78	10	12	1,16
17	S Becker	Hartswater	Northern Cape	I	Maize	None	Conventional	6,82	89	3	8	0,41
18	H Gouws	Middelburg	Mpumalanga	I	Wheat, maize, oat	Oxamyl	Disc + chisel	6,07	80	6	14	0,55
19	H Prinsloo	Kendal	Mpumalanga	R	Soybean, maize	None	Disc + chisel	6,59	81	3	16	0,59
20	P du Plessis	Bapsfontein	Gauteng	I	Potato, maize	None	Rip + disc	5,67	68	7	25	1,06
21	CWentzel	Marble Hall	Limpopo	I	Tobacco, lettuce, maize	Fenamiphos	Rip + disc	6,43	88	2	10	0,31
22	N Botha	Groblersdal	Limpopo	I	Maize, soybean, pea	None	Disc + chisel	7,36	58	9	33	1,14
23	K Human	Hartswater	Northern Cape	I	Barley, peanut, maize	Terbufos	Chisel	7,21	90	2	8	0,42
24	M Allan	Middelburg	Mpumalanga	I	Maize, soybean	None	Rip	5,21	80	5	15	0,81
25	D Berg	Leeudoringstad	North West	R	Maize	None	Conventional	7,41	96	0	4	0,20
26	A Fourie	Christiana	North West	I	Potato, maize, wheat	Terbufos	Rip	6,63	90	1	9	0,31
27	K le Roux	Carolina	Mpumalanga	R	Soybean, maize	None	Rip	6,32	83	7	10	0,90

Table 2.1 continues

Sample ID	Name of farm or producer	Town	Province	Rain fed (R) or Irrigation (I)	Crop history	Nematici-des used	Tillage practice	pH (H_2O) 1:2:5	Sand %	Silt %	Clay %	C %
28	P Roux	Viljoenskroon	Free State	R	Maize	None	Conventional	6,72	88	2	10	0,43
29	G Mulder	Hendrina	Mpumalanga	I	Potato, maize	None	Rip + disc	7,73	80	6	14	0,50
30	C Muller	Frankfort/Reitz	Free State	I	Maize	Avicta® 500FS	Rip	5,75	91	3	6	0,45
31	M van Rooyen	Warden	Free State	R	Maize, sugar bean	None	Plough + disc	5,68	72	8	20	0,50
32	J Grobler	Viljoenskroon	Free State	R	Maize	None	Conventional	5,83	94	0	6	0,21
33	J Basson	Sannieshof	North West	R	Maize, sunflower	None	No-till	5,51	78	3	19	0,37
34	J van Dyk	Middelburg	Mpumalanga	R	Maize	None	Conventional	6,87	84	4	12	0,71
35	S Bosman	Petrus Steyn	Free State	R	Maize	None	Conventional	5,12	72	8	20	0,85
36	H Wapenaar	Reitz	Free State	R	Maize, soybean	None	No-till	5,76	70	8	22	0,92
37	G Cronje	Bultfontein	Free State	I	Sunflower, maize	Oxamyl	No-till	6,66	90	0	10	0,31
38	N Leslie	Warden	Free State	R	Maize, sugar bean	None	Disc + chisel	5,95	67	9	24	0,84
39	G Cronje	Bultfontein	Free State	R	Sunflower, maize	None	Ripped	6,88	90	2	8	0,35
40	J du Randt	Bethlehem	Free State	R	Potato, sugar bean, soybean	Avicta® 500FS	Ripped	5,65	78	6	16	0,55
41	AC van Wyk	Hoopstad	Free State	R	Maize	None	Ripped	6,24	90	2	8	0,40
42	H Conradie	Grootpan	North West	I	Maize, wheat	None	Disc + chisel	7,37	73	7	20	0,85
43	J Suurd	Lichtenburg	North West	R	Maize	None	Conventional	6,39	84	1	15	0,29
44	J du Plessis	Schweizer-Reneke	North West	R	Maize	None	Conventional	6,62	91	1	8	0,20
45	P Ferreira	Bultfontein	Free State	I	Maize	None	Conventional	6,41	90	2	8	0,30
46	J Minnaar	Bothaville	Free State	R	Maize, sunflower, wheat	Terbufos, Avicta® 500FS	Rip	7,19	94	1	5	0,33
47	L Deale	Vrede	Free State	R	Soybean, maize	None	Chisel	6,75	76	8	16	0,77
48	P Becker	Warden	Free State	R	Potato, maize	Terbufos	Plough	5,50	82	6	12	0,54
49	D Schoeman	Delmas	Mpumalanga	R	Maize, bean	None	Disc + chisel	5,82	64	8	28	0,90
50	K du Preez	Grootpan	North West	R	Maize	None	Conventional	6,33	74	6	20	0,56
51	C Cronje	Vrede	Free State	I	Maize	None	Minimum till	6,32	68	12	20	0,85
52	W Venter	Frankfort	Free State	I	Maize, grass, wheat	Avicta® 500FS	Chisel	6,34	82	8	10	0,93

Table 2.1 continues

Sample ID	Name of farm or producer	Town	Province	Rain fed (R) or Irrigation (I)	Crop history	Nematicides used	Tillage practice	pH (H_2O) 1:2:5	Sand %	Silt %	Clay %	C %
53	L van Wyk	Sannieshof	North West	R	Maize	None	Conventional	6,66	83	1	16	0,26
54	J Basson	Sannieshof	North West	I	Maize, sunflower	None	No-till	7,97	76	4	20	0,43
55	F van der Merwe	Hartswater	Northern Cape	I	Maize	None	Conventional	7,28	79	5	16	0,73
56	K du Preez	Grootpan	North West	I	Maize	None	Conventional	7,20	72	6	22	0,77
57	D du Toit	Douglas	Northern Cape	I	Maize	Fenamiphos	Conventional	7,55	92	2	6	0,40
58	P du Plessis	Bapsfontein	Gauteng	R	Grass, maize	None	Rip + disc	5,16	66	9	25	1,01
59	C Cronje	Vrede	Free State	R	Soybean, maize	None	Minimum till	5,55	72	10	18	1,61
60	D Cronje	Kriel	Mpumalanga	R	Soybean, maize	None	Minimum till	5,42	81	5	14	0,41
61	H de Beer	Middelburg	Mpumalanga	R	Maize	Dagutat microbes	Rip + disc	5,55	67	7	26	1,06
62	P Ferreira	Bultfontein	Free State	R	Maize	None	Conventional	6,18	93	1	6	0,32
63	D Oosthuizen	Reitz/Bethlehem	Free State	R	Wheat, maize	Ortophos	Minimum till	6,30	88	4	8	0,28
64	C Opperman	Middelburg	Mpumalanga	R	Maize	None	Conventional	5,90	70	10	20	1,56
65	J Fourie	Bothaville	Free State	R	Sunflower, maize	Ortophos	Rip	6,66	91	1	8	0,23
66	D Viljoen	Bothaville	Free State	R	Maize	Avicta® 500FS, terbufos	Rip	6,32	90	1	9	0,28
67	H Muller	Bethlehem	Free State	R	Maize	None	Conventional	5,25	76	6	18	0,57
68	K Muller	Frankfort	Free State	R	Maize	None	Conventional	7,00	82	5	13	0,61
69	B van Wyk	Sannieshof	North West	R	Maize	None	Plough	5,61	86	2	12	0,29
70	P Carrol	Lichtenburg	North West	R	Maize, sunflower	Lamdax	No-till	5,57	76	4	20	0,49
71	F van Rooyen	Lichtenburg	North West	R	Maize	terbufos	Rip	6,69	89	0	11	0,18
72	JP Meintjies	Viljoenskroon	Free State	R	Maize	Terbufos, Avicta® 500FS	Conventional	5,12	94	0	6	0,27
73	J Potgieter	Bothaville	Free State	R	Maize	Avicta® 500FS	Rip	6,97	96	0	4	0,31
74	C Muller	Frankfort/Reitz	Free State	R	Maize, potato, oat	Avicta® 500FS	Rip	8,01	94	2	4	0,38
75	W Venter	Frankfort	Free State	R	Maize, soybean	Avicta® 500FS	Chisel	4,96	80	8	12	0,41

Table 2.1 continues

Sample ID	Name of farm or producer	Town	Province	Rain fed (R) or Irrigation (I)	Crop history	Nematicides used	Tillage practice	pH (H_2O) 1:2:5	Sand %	Silt %	Clay %	C %
76	D Myburg	Kroonstad	Free State	R	Maize	None	Conventional	4,93	78	6	16	0,42
77	A du Preez	Grootpan	North West	I	Maize, potato, soybean	None	Disc	7,59	76	4	20	0,27
78	H Prinsloo	Bultfontein	Free State	R	Maize	None	Conventional	6,39	92	0	8	0,22

Table 2.2 Average rainfall and temperature figures for the 78 maize fields sampled for a nematode survey during the 2013/14 growing season.

Sample ID	Rainfall (mm)	Average minimum temperature (°C)	Sample ID	Rainfall (mm)	Average minimum temperature (°C)
1	400	17,6	**40**	500	14,4
2	700	16,8	**41**	400	17,2
3	450	14,8	**42**	225	16,9
4	500	14,8	**43**	580	16,9
5	250	17,6	**44**	300	17,2
6	300	17,2	**45**	320	16,3
7	250	17,6	**46**	280	16,7
8	460	15,5	**47**	850	14,8
9	430	14,8	**48**	400	14
10	300	17,2	**49**	350	15,7
11	300	17,2	**50**	320	16,9
12	800	15,5	**51**	500	14,8
13	550	15,7	**52**	660	14,6
14	736	14	**53**	600	17,4
15	350	15,7	**54**	613	17,4
16	550	14,3	**55**	220	18,6
17	200	18,6	**56**	320	16,9
18	300	15,5	**57**	150	18,7
19	350	15,7	**58**	300	15,7
20	300	15,7	**59**	500	14,8
21	487	19,9	**60**	600	15,5
22	360	19,8	**61**	450	15,5
23	200	18,6	**62**	320	16,3
24	460	15,5	**63**	700	14,5
25	280	17,2	**64**	440	15,5
26	250	17,6	**65**	380	16,7
27	499	14,9	**66**	430	16,7
28	313	16,7	**67**	490	14,4
29	450	14,8	**68**	630	14,6
30	700	14,6	**69**	600	17,4
31	500	14	**70**	600	16,9
32	330	16,7	**71**	500	16,9
33	613	17,4	**72**	330	16,7
34	450	15,6	**73**	260	16,7
35	480	14,3	**74**	700	14,6
36	700	14,5	**75**	660	14,6
37	460	16,3	**76**	300	16,6
38	430	14	**77**	320	16,9
39	310	16,3	**78**	300	16,3

2.2.2. Nematode extractions and counts

Nematodes were extracted from maize roots (5 g and 50 g) and 200 g soil samples using standard techniques as described below.

2.2.2.1 Roots (5 g and 50 g)

2.2.2.1.1 The adapted centrifugal-flotation method for the extraction of a wide range of plant-parasitic nematodes from plant roots

This method is based on the gravity of nematodes and was followed according to the centrifugal-flotation method for plant material described in Kleynhans *et al.* (1996). A washed 5 g root sample, cut into approximately 0.5-1-cm pieces, was added to 250 ml tap water and shredded in a domestic kitchen blender at a high speed for 90 seconds. The root suspension was then washed through a 710 µm-aperture sieve that was placed on top of a 25 µm-aperture sieve, using a gentle stream of running tap water. The residue left on the 25 µm-aperture sieve was collected and decanted into a 50 ml centrifuge tube. Next, 2 cm^3 kaolin was added to the water suspension containing the nematodes. Kaolin is a clay mineral that forms a layer and seals off the nematodes at the bottom of the centrifuge tube (Coolen & D'Herde, 1972). The content in the centrifuge tube was then centrifuged at 1 800 rpm for 3 minutes and the supernatant decanted and disposed of. The centrifuge tube, with the nematodes and plant material sealed off at the bottom, was next filled with a sucrose solution (624 g sugar / 1000 ml water), the content was stirred well to break the kaolin layer and enable the nematodes to get into suspension. The tube was then centrifuged again at 1 800 rpm for 1 minute only. This was done to avoid exposing the nematodes in the sucrose solution too long, because they could become distorted. The supernatant was then decanted on a 25 µm-aperture sieve and rinsed thoroughly using a gentle stream of tap water. The nematodes were washed from the 25 µm-aperture sieve into a 100 ml plastic container for examination.

2.2.2.1.2 Adapted sodium hypochlorite (NaOCl) method for the extraction of *Meloidogyne* eggs and second-stage juveniles (J2)

The adapted NaOCl method, as published by Riekert (1995), has been developed specifically for the extraction of *Meloidogyne*. eggs and J2 from roots. The NaOCl solution dissolves the gelatinous matrix that surrounds the eggs and in this way releases the eggs and J2 (if present) for counting. The method is described below.

The entire root system from each maize plant was used for extraction of *Meloidogyne* eggs and J2. The roots were cut into 1 cm pieces and shaken for 4 minutes in a 1 % NaOCl solution. The solution containing the roots were decanted onto a range of nested aperture-sieves (order from top to bottom: 250 µm; 75 µm, 63 µm and 20 µm) and rinsed with running water for four minutes. The eggs and J2 of each sample were then collected in a 100 ml sampling bottle for counting.

2.2.3 Soil (200 g)

2.2.3.1 The adapted decanting and sieving, followed by the adapted sugar-flotation method

This method was followed according to the sieving-centrifugal-flotation method described by Jenkins in Kleynhans *et al.* (1997), but adapted to a certain extent. A 200 g soil sample was washed though a 2 mm-aperture sieve into a 5 l container until the latter was filled. The suspension was stirred, allowed to settle for 30 seconds and decanted through a 25 µm-aperture sieve. This process was repeated twice (representing the adapted part referred to above) with only the settling times shortened to 20 seconds and then 10 seconds, respectively. The residue left on the 25 µm aperture sieve was then transferred to a 50 ml centrifuge tubes and centrifuged for 7 minutes at 1 800 rpm. The supernatant was then carefully poured off and replaced with a sucrose solution of 624 g per liter water and shaken until the sediment and sucrose solution was mixed. The solution was then centrifuged for only three minutes at 1 800 rpm, because the nematodes could become distorted if left too long in the sucrose solution. It was then rinsed through a 25 µm-aperture sieve and the residue was collected in a 100 ml sample bottle for examination.

Nematode individuals from 5 g roots and 200 g soil, and root-knot nematode eggs and J2 from 50 g roots were counted using a De Grisse counting dish (De Grisse, 1969) and a stereo microscope (Nikon SMZ1500) (45× magnification). Nematode individuals of all genera, except *Meloidogyne*, were identified to genus level only. Thirty to 40 eggs and/or J2 from each of the 78 root-knot nematode sampled fields were obtained (described in Paragraph 2.2.2.1.2), transferred to individual Eppendorf tubes and frozen at -20 °C. Next, deoxyribonuclease (DNA) extraction was done from eggs and J2 present in each tube and molecular techniques applied for species identification as described in Paragraph 2.3 below.

2.3. Molecular identification of *Meloidogyne* spp.

2.3.1 Extraction of deoxyribonuclease (DNA) and polymerase chain reaction (PCR)

The eggs and/or J2 of the *Meloidogyne* populations obtained from each field were isolated with a micro pipette (eggs) or fished out with a fine-tip needle (J2) from the respective samples after counting. Isolation of these life stages was done using a stereo-microscope (40 ˣ magnification). The eggs and/or J2 from each individual sample were transferred to a 1.5 ml Eppendorf tube containing approximately 20 µl, double-distilled water. The DNA of the eggs/J2 from each field was extracted seperately using the following procedure: First, the nematodes were crushed using a melted, glass pipette tip. After this a 20 µl chelex®100 (5 %) solution and 3 µl proteinase K (20 mg/ ml) was added to each sample. The J2 homogenate was then centrifuged at 12 000 rpm for 20 seconds and incubated at 56 °C for 2 hours. It was then further incubated at 95 °C for 10 minutes to deactivate the proteinase K and stored at -20 °C until it was used for the polymerase chain reaction (PCR).

Two approaches was used to identify *Meloidogyne* spp. present in each sample which included the amplification and sequencing of the mitochondrial DNA (mtDNA), NADH dehydrogenase subunit 5 gene (NADH5) and amplification of a specific region of ribosomal DNA (rDNA) with species-specific primers in SCAR-PCR. The techniques are described in Paragraphs 2.3.1.1 and 2.3.1.2, respectively.

2.3.1.1 NADH dehydrogenase subunit 5 (NADH5) technique and deoxyribonuclease (DNA) sequencing

The DNA homogenate of eggs/J2 from each field that were contained in individual PCR tubes were prepared for sequencing by adding 12.5 µl Master mix, 8 µl nuclease-free water, 1 µl forward primer, 1 µl reverse primer and 8 µl template DNA. The primers were diluted to 10 p.mol before use. All samples were centrifuged for 10 seconds and placed in the C1000™ Thermal Cycler (BioRad) under the conditions described in Table 2.3. The PCR product was then analyzed by electrophoresis on a 1.5 % agarose gel with 1x TAE buffer. An O'GeneRuler™ 1kb DNA Ladder was loaded in the first well to establish the size of the DNA bands for the eggs/J2 present in each sample. Next, 4 µl template DNA of the eggs/J2 from each field was mixed with 2 µl GelRed and added to individual wells in the agarose gel. The gels were then ran for 30 minutes at 120 V in an electrophoresis chamber and visualised with ultraviolet (UV) illumination. The size of the NADH5 band was visible at the 610 bp fragment length. The remaining DNA template of eggs/J2 from each field was sent to

the South African sequencing company, inqababiotec™ (http://www.inqababiotec.co.za) in Pretoria, for sequencing. Phylogenetic analysis was done by aligning the sequences with Clustal Wusing the Maximum Likelihood method (1 000 bootstrap values) as implemented in Mega7 software (Kumar *et al*., 2016). *Aphelenchoides besseyi* (Accession no. KJ739799) was selected from Genbank and used as the outgroup.

2.3.1.2 Sequence-derived amplified region – polymerase chain reaction (SCAR-PCR)

Specific primers are commercially available to amplify the DNA of *Meloidogyne* spp. life stages. Primer sets are available for species such as *M. arenaria*, *Meloidogyne chitwoodi* Golden, O'Banon, Santo & Finley, 1980, *Meloidogyne enterolobii* Yang & Eisenbach, 1983, *Meloidogyne fallax* Karssen, 1996, *Meloidogyne hapla* Chitwood, 1949, *M. incognita* and *M. javanica*. The primers each constitute of eight to ten nucleotides (Table 2.3) (Blok & Powers, 2009). For this specific study, SCAR-PCR assays were done only to determine the presence of *M. arenaria, M. enterolobii, M. incognita and M. javanica* since NADH5 analyses was done first and indicated the presence of only these species. The egg/J2 DNA from each respective field were subjected to the following protocol: each PCR tube contained 12.5 µl Master mix, 8.5 µl nuclease-free water, 1 µl forward primer, 1 µl reverse primer and 2 µl template DNA. The primers were diluted to 10 p.mol before use. A 'no template' control (NT- no DNA, only containing nuclease free water) and a known reference sample containing DNA of monoculture populations of each *Meloidogyne* spp. were also included in the SCAR-PCR amplification process (see Table 2.4). The content of the PCR tubes were then centrifuged and placed in the C1000™ Thermal Cycler (BioRad) under the conditions described in Table 2.3. The products were then analysed through electrophoresis as described in paragraph 2.3.1.1.

The reference *M. incognita* and *M. javanica* monoculture populations used in this study were reared *in vivo* at the Nematology Laboratory of the North-West University, Potchefstroom. These reference species populations were earlier identified with the SCAR-PCR technique (Fourie *et al*., 2012). Life stages of the other reference species used, *viz*. *M. arenaria* and *M. enterolobii*, were obtained from Prof. Gerrit Karssen from the Netherlands, Food and Consumer Product Safety Authority, Ministry of Economic Affairs, Hc Wageningen. The DNA from 100 J2 from these respective populations was extracted to confirm the identity of these species should they be present in the samples (See table 2.4).

Table2.2 Primer codes used for the identification of *Meloidogyne* spp. for both NADH5 and SCAR-PCR.

Code	Primer sequence 5'-3'	Specificity and reference source
[1]FNAD5	TATTTTTTGTTTGAGATATATTAG	*Meloidogyne* spp. identification (mtDNA gene) Janssen *et al.*(2016)
[2]RNAD5	CGTGAATCTTGATTTTCCATTTTT	
[1]Far	TCGGCGATAGAGGTAAATGAC	*Meloidogyne arenaria*-specific SCAR; Zijlstra *et al*. (2000)
[2]Rar	TCGGCGATAGACACTACAACT	
[1]Finc	CTCTGCCCAATGAGCTGTCC	*Meloidogyne incognita*-specific SCAR; Zijlstra *et al*. (2000)
[2]Rinc	CTCTGCCCTCACATTAGG	
[1]Fjav	GGTGCGCGATTGAACTGAGC	*Meloidogyne javanica*-specific SCAR; Zijlstra *et al*. (2000)
[2]Rjav	CAGGCCCTTCAGTGGAACTATAC	
[1]Fent	AACTTTTGTGAAAGTGCCGCTG	*Meloidogyne enterolobii*-specific SCAR; Long *et al.* (2006)
[2]Rent	TCAGTTCAGGCAGGATCAACC	

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

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

Amplification conditions	*M. arenaria*	*M. enterolobii*	*M. incognita*	*M. javanica*	NADH5
Denaturation temperature	94 °C, 2'	94 °C, 2'	94 °C, 2'	94 °C, 2'	94 °C, 2'
No. of cycles	35	35	35	35	40
Denaturation temperature	94 °C, 30"	94 °C, 30"	94 °C, 30"	94 °C, 30"	94 °C, 60"
Annealing temperatures	61 °C, 30"	64 °C, 30"	55 °C, 30"	64 °C, 30"	45 °C, 60"
Extension temperatures	72 °C, 60"	72 °C, 60"	72 °C, 60"	72 °C, 60"	72 °C, 180"
Final extension temperatures	72 °C, 5'	72 °C, 5'	72 °C, 5'	72 °C, 5'	72 °C, 10'

Table 2.4 *Meloidogyne* spp. populations used as references during the identification of root-knot nematode species contained in maize roots sampled from 78 fields during 2013/14.

***Meloidogyne* spp. and life stage**	**Host and country of origin**	**DNA fragment size (bp)**
Meloidogyne arenaria; second-stage juveniles (J2)	*Echinocactus grusonni* (Golden Barrel Cactus); The Netherlands	420
Meloidogyne enterolobii second stage juveniles	*Psidium guajava* (Guava), Mbombela, Mpumalanga Province, South Africa	200
Meloidogyne incognita; mature females	*Zea mays* (maize); Vryburg Northern-Cape Province, South Africa	1 200
Meloidogyne javanica; mature females	*Cucurbita pepo* (Pumpkin); Kuruman Correctional Center, Northern Cape Province, South Africa	670

2.4 Data analyses

Prominence values (PVs), used to determine the frequency of occurrence and mean population densities of nematodes associated with crops (De Waele & Jordaan, 1988; Fourie *et al*., 2001) were calculated for nematode genera identified during this study. This parameter represents an index to categorize nematode genera/species that were identified during surveys. High prominence values, therefore, in this study indicate which nematode genera/species were predominant in root and soil samples from maize for each locality. Conversely, low PVs indicate nematode genera/species of minor importance to maize according to this study.The equation used to calculate PV is as follows:

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

- 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.

2.5. Results

2.5.1 Nematode data

2.5.1.1 Roots (50g)

Table 2.5 Prominence values (PVs), frequency of occurrence (% FO) and mean population densities (MPD) of *Meloidogyne* spp. per 50 g maize roots for 78 fields sampled during the 2013/14 growing season.

Sample ID	PV	% FO	MPD	Sample ID	PV	% FO	MPD
37	38 479	100	38 479	**72**	109	25	219
57	11 463	100	11 463	**46**	91	60	118
69	9 867	100	9 867	**32**	87	50	123
11	8 094	100	8 094	**35**	84	38	136
17	7 836	100	7 836	**2**	80	63	101
71	5 775	100	5 775	**42**	75	38	123
39	5 084	100	5 084	**10**	70	13	193
15	3 006	100	3 006	**24**	69	63	88
7	2 160	88	2 302	**43**	65	50	92
55	2 087	100	2 087	**30**	57	38	92
20	2 043	100	2 043	**76**	56	50	79
19	1 973	100	1 973	**65**	51	60	66
23	1 720	100	1 720	**28**	40	25	79
26	1 694	75	1 956	**44**	40	25	79
73	1 614	100	1 614	**53**	39	38	62
63	892	63	1 129	**77**	38	63	48
6	879	75	1 015	**74**	35	38	57
45	831	75	959	**50**	29	38	48
29	636	63	805	**64**	26	25	53
48	563	63	709	**13**	25	13	70
1	543	88	578	**36**	25	38	40
68	535	75	617	**49**	25	13	70
25	534	88	569	**75**	22	25	44
41	523	63	657	**22**	21	25	35
60	459	63	581	**70**	20	38	31
27	364	88	389	**4**	19	38	31
62	329	88	350	**5**	16	25	31
3	322	63	407	**59**	11	25	22
66	288	75	333	**31**	11	13	31
18	276	100	276	**8**	7	25	13
34	245	88	263	**52**	7	13	18
21	232	63	293	**56**	6	13	18
33	179	75	206	**51**	0	0	0
9	177	63	224	**61**	0	0	0
58	172	88	184	**67**	0	0	0
54	164	75	189	**14**	0	0	0
12	156	88	166	**16**	0	0	0
78	129	63	162	**47**	0	0	0
40	125	25	249	**38**	0	0	0

Meloidogyne spp. eggs and J2 were present in 50 g maize roots from 91 % of the fields sampled (Table 3.5). Substantial variation existed in terms of the abundance and occurrence of *Meloidogyne* spp. for the 78 fields.

The MPDs for *Meloidogyne* spp. ranged between zero,for seven of the samples and a high 38 479 (Sample 37) (Table 2.5). At seven of the fields *Meloidogyne* eggs and J2 were absent (0 % occurrence) from the root samples. For the majority of the fields, root-knot nematode eggs and J2 were present in 13 % (samples 10, 13, 31, 49, 52, 56) to 100 % (14 of the samples) of the samples analysed from each field.

3.5.1.2 Roots (5g)

3.5.1.2.1 *Meloidogyne* spp.

Table 2.6 Prominence values (PVs), frequency of occurrence (% FO) and mean population densities of *Meloidogyne* spp. per 5 g maize roots for 78 fields sampled during the 2013/14 growing season.

Sample ID	PV	% FO	MPD	Sample ID	PV	% FO	MPD
37	998	100	998	**64**	1	13	4
25	238	88	254	**4**	1	13	4
67	140	100	140	**18**	1	13	4
28	133	75	153	**16**	1	13	4
39	127	100	127	**5**	1	13	4
11	125	75	144	**8**	1	13	4
17	99	88	105	**22**	1	13	4
10	63	63	79	**62**	1	13	4
15	63	63	79	**51**	0	0	0
32	57	100	57	**30**	0	0	0
78	52	63	66	**1**	0	0	0
19	49	38	79	**60**	0	0	0
65	40	25	79	**12**	0	0	0
66	38	75	44	**63**	0	0	0
23	38	75	44	**55**	0	0	0
69	35	63	44	**71**	0	0	0
76	31	50	44	**3**	0	0	0
44	25	50	35	**29**	0	0	0
73	22	50	31	**42**	0	0	0
35	19	38	31	**61**	0	0	0
26	16	13	44	**36**	0	0	0
33	16	38	26	**40**	0	0	0
68	16	38	26	**46**	0	0	0
49	14	38	22	**43**	0	0	0
57	13	25	26	**34**	0	0	0
27	11	38	18	**72**	0	0	0
9	9	25	18	**56**	0	0	0
6	9	25	18	**13**	0	0	0
77	7	25	13	**47**	0	0	0
41	7	13	19	**24**	0	0	0
50	7	25	13	**31**	0	0	0
74	5	13	13	**38**	0	0	0
7	5	25	9	**48**	0	0	0
59	3	13	9	**70**	0	0	0
21	3	13	9	**20**	0	0	0
54	3	13	9	**58**	0	0	0
14	3	13	9	**45**	0	0	0
53	3	13	9	**52**	0	0	0
2	1	13	4	**75**	0	0	0

Meloidogyne spp. eggs and J2 were present in 5 g maize roots from 40 % of the fields sampled (Table 2.6). Substantial variation existed in terms of the abundance and occurrence of *Meloidogyne* spp. for the 78 fields.

The PVs for *Meloidogyne* spp. ranged between zero for 31 of the samples and 998 (Sample 37), and the MPDs between zero (Sample 75) and 998 (Sample 37) (Table 2.6). For the rest of the fields, root-knot nematodes were present in 13 % (17 of the samples) to 100 % (Samples 32, 37, 39, 67) of the samples analysed from each field.

2.5.1.2.2 *Pratylenchus* spp.

Table 2.7. Prominence values (PVs), frequency of occurrence (% FO) and mean population densities of *Pratylenchus* spp. per 5 g maize roots for 78 fields sampled during the 2013/14 growing season.

Sample ID	PV	% FO	MPD	Sample ID	PV	% FO	MPD
33	899	88	958	**62**	97	100	97
60	490	100	490	**6**	95	88	101
61	442	100	442	**64**	90	88	96
53	419	100	419	**12**	83	88	88
76	389	100	389	**34**	83	100	83
78	385	100	385	**52**	76	75	88
24	372	100	372	**65**	74	100	74
41	350	100	350	**46**	74	100	74
45	324	100	324	**75**	72	75	83
15	306	100	306	**27**	68	75	79
74	295	75	341	**3**	62	50	88
10	293	100	293	**23**	61	75	70
9	280	100	280	**68**	61	75	70
54	263	100	263	**25**	57	88	61
63	236	100	236	**5**	56	50	79
50	236	100	236	**31**	53	75	61
42	201	100	201	**71**	49	63	62
13	201	100	201	**36**	48	63	61
37	193	100	193	**47**	48	63	61
39	193	88	206	**66**	46	25	92
69	188	100	188	**8**	45	75	53
43	188	100	188	**38**	43	50	61
56	184	100	184	**44**	42	63	53
4	166	100	166	**77**	41	88	44
70	164	88	175	**26**	38	75	44
28	164	88	175	**57**	38	75	44
29	162	100	162	**22**	37	50	53
73	162	100	162	**1**	34	50	48
19	149	100	149	**17**	34	75	39
11	144	88	153	**7**	33	88	35
59	140	100	140	**30**	28	63	35
20	127	100	127	**51**	25	50	35
40	123	100	123	**32**	21	63	26
58	119	88	127	**48**	15	50	22
35	118	100	118	**55**	11	25	22
49	115	88	123	**18**	9	25	18
2	114	63	144	**72**	9	25	18
16	114	100	114	**67**	1	13	4
14	109	100	109	**21**	0	0	0

Pratylenchus spp. eggs and J2 were present in 5 g maize roots from 99 % of the field,this will be extremely puzzling especially as the samples were taken at end of growing seasons sampled (Table 2.7). Substantial variation existed in terms of the abundance and occurrence of *Pratylenchus* spp. for the 78 fields.

The PVs for *Pratylenchus* spp. ranged between zero for one of the samples (Sample 21) and 899 (Sample 33), and MPDs between zero (Sample 21) and 958 (Sample 33) (Table 2.7). For the majority of the fields, lesion nematodes were present in 13 (Sample 67) to 100 % (34 of the samples) of the samples analysed from each field.

2.5.1.3 Soil (200g)

Table 2.8 Prominence values (PVs)of plant-parasitic nematode genera per 200 g soil samples for 78 fields sampled during the 2013/14 growing season.

Sample ID	*Mel*[1]	*Prat*[2]	*Heli*[3]	*Crico*[4]	*Roty*[5]	Sample ID	*Mel*[1]	*Prat*[2]	*Heli*[3]	*Crico*[4]	*Roty*[5]	Sample ID	*Mel*[1]	*Prat*[2]	*Heli*[3]	*Crico*[4]	*Roty*[5]
37	3 294	108	5	1	14	**9**	6	42	2	4	0	**51**	0	214	49	0	7
56	390	80	30	0	0	**8**	5	96	79	0	0	**2**	0	15	7	0	0
55	285	91	47	0	0	**28**	5	14	14	2	0	**64**	0	18	256	0	106
43	215	128	25	23	21	**17**	5	52	1	3	0	**60**	0	245	551	9	306
71	205	220	47	8	9	**15**	4	55	89	1	1	**12**	0	34	22	1	4
57	187	16	2	0	2	**78**	4	78	71	45	29	**49**	0	26	132	0	278
39	167	101	0	21	475	**38**	4	328	555	0	1	**66**	0	22	5	6	0
77	116	76	55	0	0	**30**	3	25	1	7	0	**4**	0	84	53	1	35
23	103	59	37	0	0	**73**	3	5	0	174	0	**29**	0	57	4	0	0
48	85	88	64	0	15	**72**	3	9	36	28	0	**61**	0	52	493	0	261
11	69	78	55	3	6	**41**	2	63	13	124	2	**18**	0	9	14	1	0
19	62	62	92	0	0	**54**	2	94	23	6	3	**67**	0	396	103	3	0
25	51	90	6	0	0	**16**	2	67	82	0	38	**36**	0	64	51	0	100
74	50	99	9	25	3	**47**	2	49	9	0	1	**14**	0	53	46	0	0
69	45	304	51	7	2	**24**	2	68	91	0	0	**44**	0	63	39	30	1
20	32	205	70	0	0	**31**	2	210	161	1	21	**65**	0	12	0	72	0
7	28	54	1	0	0	**10**	1	22	48	0	22	**32**	0	109	23	1	0
1	23	34	1	1	0	**59**	1	397	59	3	28	**46**	0	9	0	73	0
27	15	31	90	0	3	**21**	1	15	10	1	0	**5**	0	8	18	0	0
3	14	38	108	1	2	**76**	1	59	9	0	432	**13**	0	25	153	0	23
26	13	39	3	22	0	**63**	1	465	367	1	0	**22**	0	20	9	0	0
75	11	463	195	0	0	**33**	1	157	211	9	6	**70**	0	156	75	0	232
42	9	14	7	0	0	**68**	1	28	20	2	1	**58**	0	5	17	1	0
40	7	364	89	1	0	**53**	1	253	92	3	10	**45**	0	232	2	140	0
34	6	38	165	72	15	**6**	1	62	2	1	0	**35**	0	275	196	0	32
50	6	77	33	0	0	**62**	1	116	3	93	4	**52**	0	26	23	1	3

Mel = Meloidogyne; *Prat = Pratylenchus*; *Heli= Helicotylenchus*; *Crico= Criconema*; *Roty= Rotylenchulus*

Plant-parasitic nematodes genera present in 200 g soil samples were, in order of predominance: *Meloidogyne*, *Pratylenchus*, *Helicotylenchus*, *Criconema* Hofmänner and Menzel, 1914, *Rotylenchulus* Fillipjev, 1936*, Tylenchorhynchus* and *Xiphinema* as well as individuals from the family Trichodoridae (either *Nanidorus* and/or *Paratrichodorus*) (Table 2.8).

The PVs for *Meloidogyne* ranged between zero (26 of the samples) and 3 294 (Sample 37) (Table 2.8), and MPDs between zero (20 of the samples) and 3294 (Sample 37) (Annex Table 2.10). The occurrence of root-knot nematode J2 in the samples collected ranged from zero (20 of the samples) to 100 % (Samples 37, 56, 57) (Annex Table 2.10).

The PVs (Table 2.8) and MPDs (Annex Table 2.11) for *Pratylenchus* ranged between 5 (Samples 58, 73) and 465 (Sample 63). The occurrence of lesion nematode individuals in the samples collected ranged from 50 % (Samples 13 and 73) to 100 % (39 of the samples) of the samples analysed from each field (Annex Table 2.11).

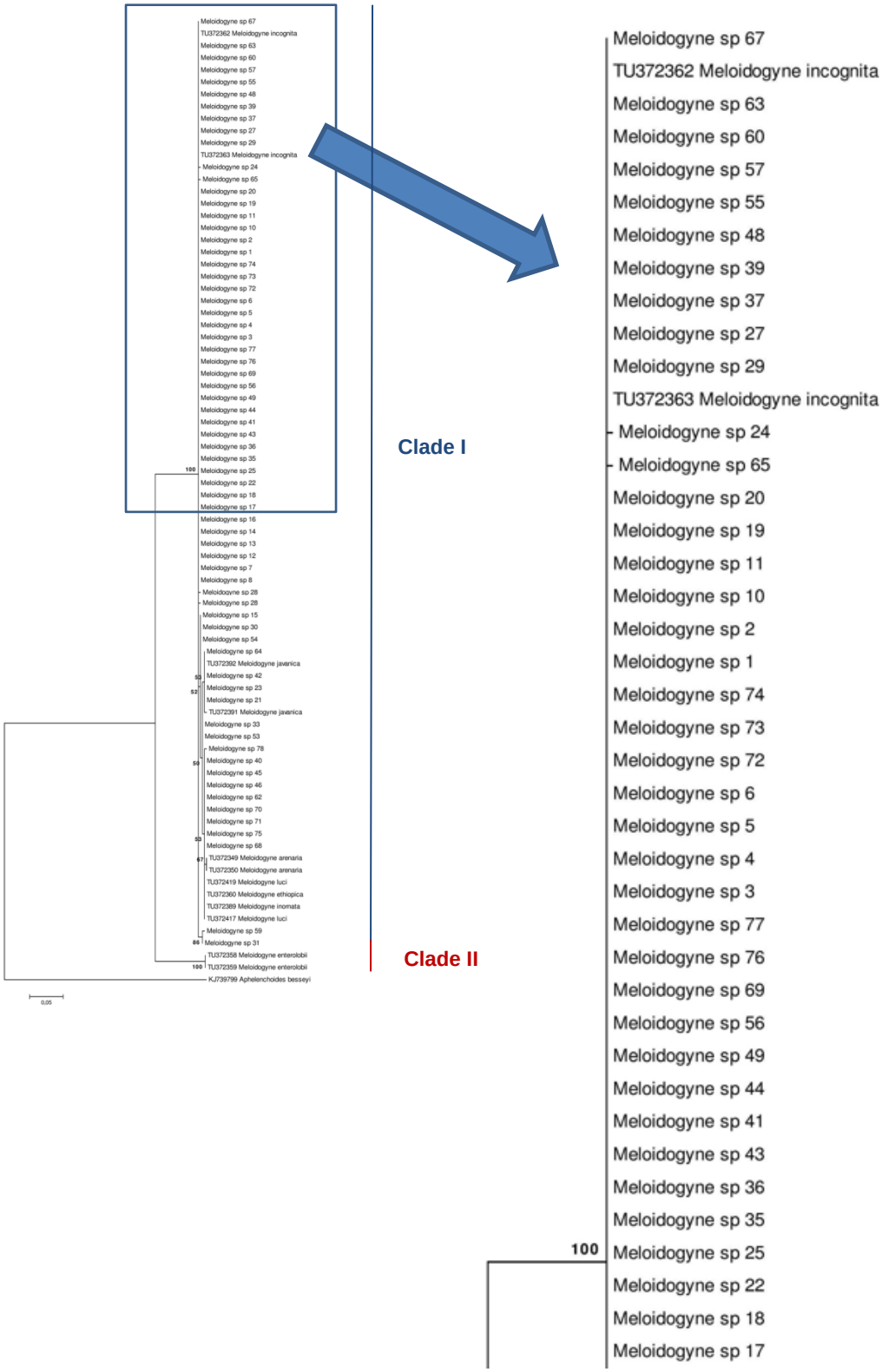
The PVs for *Helicotylenchus* ranged between zero (Samples 39, 46, 65, 73) and 555 (Sample 38) (Table 2.8) and MPDs between zero (Samples 39, 46, 65 and 73) and 593 (Sample 38) (Annex Table 2.12). The occurrence of *Helicotylenchus* individuals in samples collected ranged from zero (Samples 39, 46, 65, 73) to 100 % (19 of the samples) (Annex Table 2.12).

The PVs for the *Criconema* ranged between zero (23 of the samples) and 174 (Sample 73) (Table 2.8), and MPDs between zero (27 of the samples) and 174 (Sample 73) (Annex Table 2.13). The occurrence of ring nematodes in samples collected ranged from zero (29 of the samples) to 100 % (Sample 73) (Annex Table 2.13).

The PVs for *Rotylenchulus* ranged between zero (38 of the samples) and 475 (Sample 39) and (Table 2.8), and the MPDs between 508 (Sample 39) and 0 (33 of the samples) for the 78 fields (Annex Table 2.14).The occurrence of *Rotylenchulus* individuals in samples collected ranged from zero (33 of the samples) to 100 % (Samples 61, 64, 76) (Annex Table 2.14).Very low abundance and occurrence were recorded for *Tylenchorhynchus* and *Xiphinema*, as well as the family Trichodoridae (either *Nanidorus* and/or *Paratrichodorus*), resulting in low PVs (Annex Tables 2.15, 2.16, 2.17). Since PVs and MPDs for these genera ranged between zero and 2, 22, 8 and 9, 37, 22, respectively for these genera, no further discussion is warranted.

2.5.2 Molecular identification of *Meloidogyne* spp.

2.5.2.1 NADH dehydrogenase 5'(NADH5) technique



Meloidogyne spp. of the 68 identified from 78 maize fields sampled in South African maize production areas using ClustalW and MEGA7 software. *Aphelenchoides besseyi* (Accession no. KJ739799), selected from Genbank was used as the outgroup.

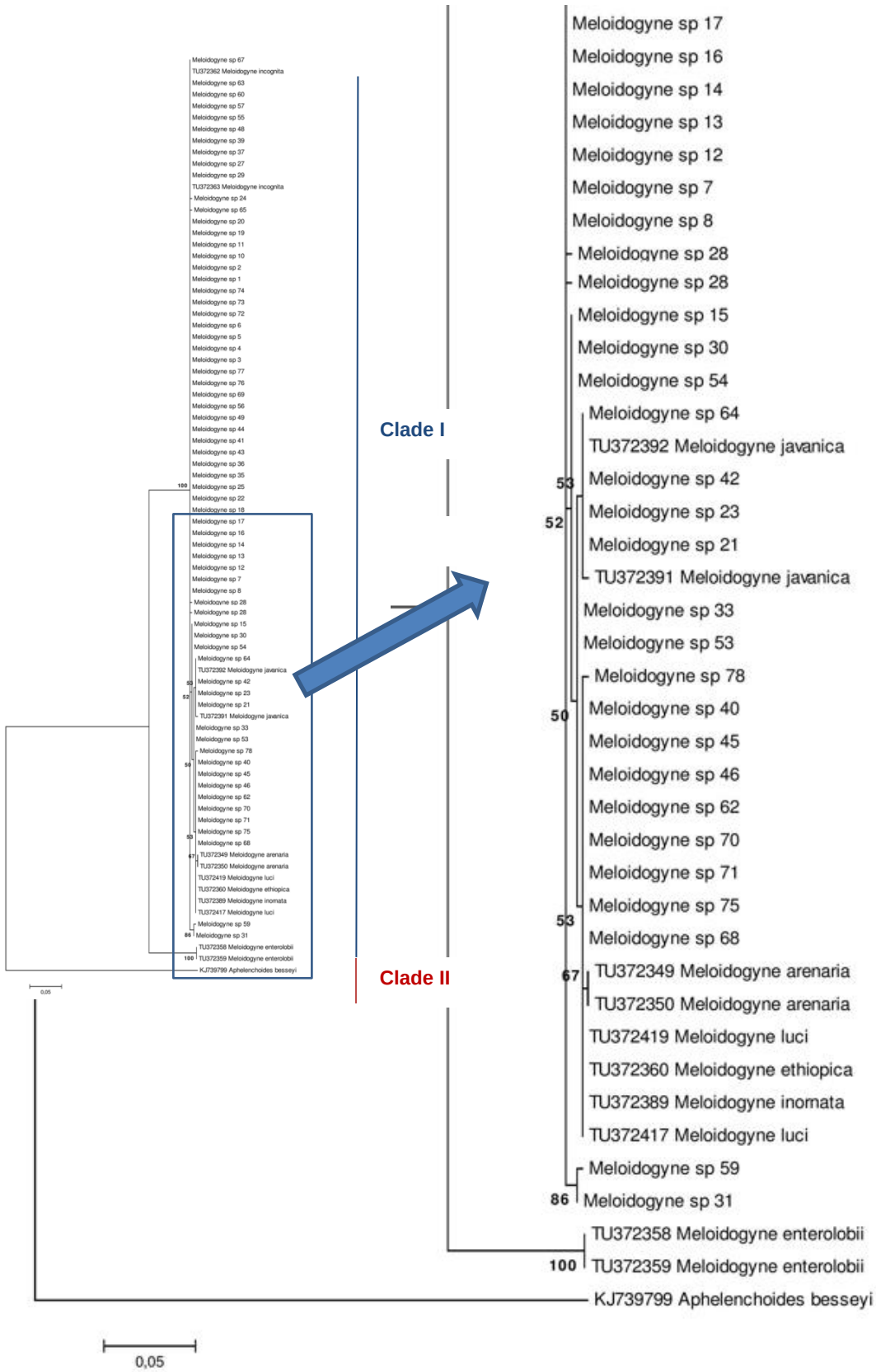


Fig.2.2A. Maximum likelihood tree of the NADH5 gene fragment sequences of 39.
Fig. 2.2 Sequences of 29 *Meloidogyne*populations of the 68 identified from 78 maize fields sampled in South African maize production areas using ClustalW and MEGA7 software.

Aphelenchoides besseyi (Accession no. KJ739799), selected from Genbank was used as the outgroup. Maximum likelihood tree of the NADH5 gene fragment.

The NADH5 DNA sequences of *Meloidogyne*. populations of only 68 of the 78 fields sampled for this study could be obtained (Figs 2.2A & B) due to problems experienced with DNA quality of the other 10 populations. According to phylogenetic analysis of these sequences the *Meloidogyne* spp. identified for this study were placed in two clades. Clade I , contained *M. arenaria*, *M. incognita*, *Meloidogyne luci* Carneiro, Correa, Almeida, Gomes, Deimi, Castagnone-Sereno & Karssen, 2014, *Meloidogyne ethiopica* Whitehead, 1968, and *Meloidogyne inomata* Lordello, 1956 populations extracted from Genbank and all 68 NADH5 sequences of the *Meloidogyne* spp. present in the 68 fields sampled during this study. Clade II contained *M. enterolobii* sequences extracted from Genbank (Figs. 2.2A & B). *Aphelenchoides besseyi* (Accession no. KJ739799) selected from Genbank was used as the outgroup.

2.5.2.2 Sequence-derived amplified region – polymerase chain reaction (SCAR-PCR)

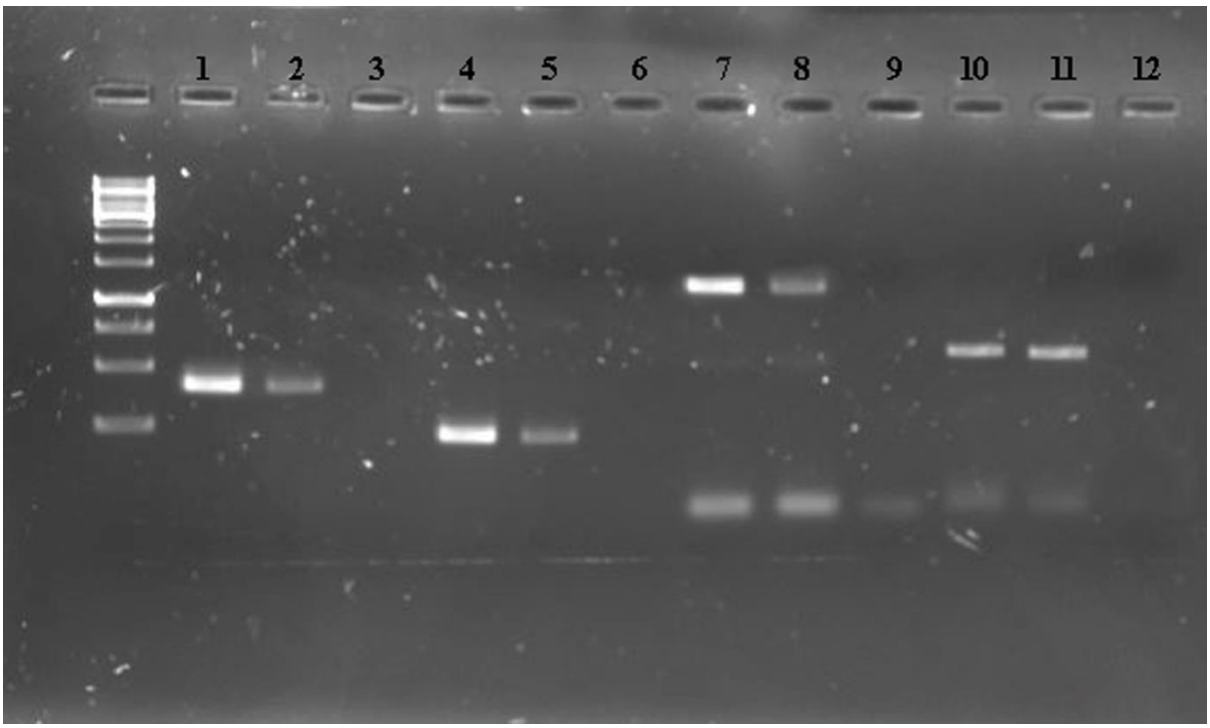


Fig. 2.3 The DNA bands of four *Meloidogyne* spp. identified from 78 maize fields sampled during the 2014/15 growing season in South African production areas with 1 = *M. arenaria* standard; 2 = *M. arenaria* from Field 11; 4 = *M. enterolobii* standard; 5 = *M. enterolobii* from Field 12; 6 = *M. incognita* standard; 7 = *M. incognita* from Field 13; 10 = *M. javanica* standard; 11 = *M. javanica* from Field 21.Wells 3, 6, 9 and 12 represented water controls containing no *Meloidogyne* DNA.

Amplification of root-knot nematode J2 DNA from seven of the 78 fields (Table 2.9) sampled produced no SCAR fragments for any of the four species-specific SCAR primers used. Problems were experienced with low quality DNA for these seven fields.

Four *Meloidogyne* spp. were identified using species-specific primers from 71 of the 78 fields sampled during this study. These include *M. arenaria*, *M. enterolobii*, *M. incognita* and *M. javanica* (Fig. 2.3; Table 2.9). Figure 2.3 is a representation of the DNA bands that were amplified for these four species using the SCAR-PCR technique. Wells 1 and 2 indicate the presence of *M. arenaria* (DNA from the standard and sample 11, respectively) with the 420bp fragment being amplified (Zijlstra *et al.*, 2000). Only two populations, Fields 11 and 68 were this way identified as containing *M. arenaria* (Table 2.9).

Wells 4 and 5 indicated the presence of *M. enterolobii* (DNA from the standard and Field 12, respectively) with the 200bp fragment being amplified (Long *et al.*, 2006) (Fig. 2.3). Only the latter population was hence identified as belonging to this species (Table 2.9).

Wells 6 and 7 show amplification of the 1 200bp fragment for *M. incognita* (Zijlstra *et al.*, 2000) from the standard and Field 13, respectively (Fig. 2.3). Forty-nine of these populations contained only *M. incognita* (Table 2.9), while 21 consisted of mixed populations (Table 2.9). The 18 fields that contained mixed populations of *M. incognita* and *M. javanica* were 1; 2; 3; 4; 5; 9; 10; 18; 22; 23; 29; 31; 42; 45; 50; 60; 64 and 73. The other mixed populations were from two fields and contained *M. incognita* and *M. arenaria* (11 and 68), while one field contained a mixture of *M. incognita* and *M. enterolobii* (12).

Wells 10 and 11 indicate the presence of *M. javanica* with the 670bp fragment being amplified (Zijlstra *et al.*, 2000) for the standard population and one from Field 21, respectively (Fig. 2.3). Only one monoculture population, from field 21, were identified as *M. javanica* populations (Table 2.9), while the other 18 fields contained mixed populations of *M. incognita* and *M. javanica* (see previous paragraph for sample numbers containing these two species).

Meloidogyne incognita was the most common species and was present in maize roots from 70 of the 71 fields, followed by *M. javanica* (19 fields), *M. arenaria* (2 fields) and *M. enterolobii* (1 field). Twenty-one (30 %) of the 71 samples consisted of mixed *Meloidogyne*

spp. populations, while the other 50 (70 %) consisted of monoculture populations of the four respective species. **Table 2.9** The identity of *Meloidogyne* spp. sampled from maize roots and soil from 78 fields during the 2013/14 growing season according to species-specific SCAR-PCR primers.

Sample ID	*Ma*[1]	*Me*[2]	*Mi*[3]	*Mj*[4]	Sample ID	*Ma*[1]	*Me*[2]	*Mi*[3]	*Mj*[4]	Sample ID	*Ma*[1]	*Me*[2]	*Mi*[3]	*Mj*[4]
1			+	+	**27**			+		**53**			+	
2			+	+	**28**			+		**54**	Not identified			
3			+	+	**29**			+	+	**55**			+	
4			+	+	**30**			+		**56**			+	
5			+	+	**31**			+	+	**57**			+	
6			+		**32**			+		**58**			+	
7			+		**33**			+		**59**			+	
8			+		**34**			+		**60**			+	+
9			+	+	**35**			+		**61**	Not identified			
10			+	+	**36**			+		**62**			+	
11	+		+		**37**			+		**63**			+	
12		+	+		**38**	Not identified				**64**			+	+
13			+		**39**			+		**65**			+	
14			+		**40**			+		**66**			+	
15			+		**41**			+		**67**			+	
16			+		**42**			+	+	**68**	+		+	
17			+		**43**			+		**69**			+	
18			+	+	**44**			+		**70**	Not identified			
19			+		**45**			+	+	**71**			+	
20			+		**46**	Not identified				**72**			+	
21				+	**47**			+		**73**			+	+
22			+	+	**48**			+		**74**			+	
23			+	+	**49**			+		**75**	Not identified			
24			+		**50**			+	+	**76**			+	
25			+		**51**	Not identified				**77**			+	
26			+		**52**			+		**78**			+	

[1]*Ma = M. arenaria*; [2]*Me = M. enterolobii*; [3]*Mi = M. incognita*;[4]*Mj = M. javanica*

2.6 Discussion

Results from this extensive survey that included 78 maize fields in local production areas confirmed that *Meloidogyne*, followed by *Pratylenchus* are the predominant nematode pest genera (Fourie *et al.,* 2017). Except for the three *Meloidogyne* spp. known to parasitise maize in South Africa (*viz*. *Meloidogyne incognita, M. javanica* and *M. arenaria* (Mc Donald *et al*., 2017; SAPPNS[1]), *M. enterolobii* has also been identified. This is the first report of this emerging and aggressive (EPPO, 2011) root-knot nematode species parasitising maize roots in South Africa. *Meloidogyne enterolobii* has been identified infecting roots of crops grown in only a few production areas such as Kwa-Zulu Natal (Onkendi & Moleleki, 2013) and Mpumalanga (Agenbag, 2016). According to a reportby the European and Mediteranean Plant Protection Organization (EPPO) (EPPO, 2014), maize is a non- or very poor host for *M. enterolobii*.

The presence of eggs and J2 of *Meloidogyne* in 91 % of the 50-g root samples obtained from the 78 maize fields sampled is a direct indication of the importance of this nematode genus in local maize production areas. Moreover, the high level of efficacy of the adapted NaOCl method (Riekert, 1995) used to specifically extract eggs of sedentary endoparasitic genera such as *Meloidogyne* (Hussey & Barker, 1973) and used widely Vovlas *et al*., 2005; Fourie *et al*., 2015; Baidoo *et al*., 2017; Marais *et al*., 2017) from crop roots has been demonstrated with this extensive study. The high abundance of *Meloidogyne* spp. eggs and J2 in maize roots examined during this survey is noteworthy and in agreement with results for this genus for conventional maize crops (Riekert, 1995; 1996; Riekert & Henshaw, 1998; Bekker *et al*., 2007; Fourie *et al*., 2015). It, however, differ from earlier reports (De Waele & Jordaan, 1988) when *Pratylenchus* was considered the economically most important genus in local maize fields. Although the extraction methods used in studies from 1995 and the survey by De Waele & Jordaan (1988), other factors could also have played a role in this anomaly regarding these two genera. According to PVs and MPDs for *Meloidogyne* per 50 g roots, the areas with the highest infestation levels of *Meloidogyne* (ranging between 5 000 and 39 000 eggs and J2 / 50 g roots) were in the Bultfontein (Fields 39 & 37), Douglas (Field 57), Sannieshof (Field 69), Makwassie (Field 11), Vaalharts (Field 17) and Lichtenburg areas (Field 71). These sites are located across the Free State, Northern Cape and North West provinces where soils with sand contents ranging between 46 to 96 % prevail. This finding is in contrast that *Meloidogyne* prefer sandy soils for optimal development and reproduction (Curtis *et al*., 2009).

Due to the extensive nature of this survey, morphological identification of *Meloidogyne* spp. was not done. Instead, molecular methods were used to elucidate the identity of the root-knot nematode species present maize fields. Use of both the SCAR-PCR (indicating both single and mixed species populations) and the NADH5 (confirming only single-species populations) techniques complemented each other to a certain extent. While the SCAR-PCR enabled accurate identification of the four *Meloidogyne* spp., *viz*. *M. arenaria, M. incognita, M. javanica* and *M. enterolobii*, NADH5 sequences for the former three species could not be distinguished from each other and were grouped in one clade. Furthermore, the presence of *M. enterolobii* as confirmed by SCAR-PCR could not be verified with the NADH5 technique. An explanation for this is that the only field that contained the latter species also contained *M. incognita*. From this mixed population it is deducted that only *M. incognita* eggs and/or J2 were unknowingly selected for NADH5 analysis while it was not the case for the SCAR-PCR. This immediately confirms the inability of the NADH5 to indicate the presence of mixed species opposed to the SCAR-PCR with which it could be done (Karssen *et al.*, 2013). Since it is not uncommon for two or more *Meloidogyne* spp. to co-exist in mixed populations (Kleynhans, 1991), results from this study that 30 % of the *Meloidogyne* spp. identified occurred in mixed populations was not unexpected. In local maize-based cropping systems, *M. incognita* and *M. javanica,* are for example often present in mixed populations (Riekert, 1996; Riekert & Henshaw, 1988; Mc Donald *et al*., 2017), with *M. arenaria* also occuring in some (Agenbag, 2016).

Meloidogyne incognita outcompeted the other three species and was present in 99 % of the fields followed by *M. javanica* (30 %), *M. arenaria* (3 %) and *M. enterolobii* (1 %). Predominance by *M. incognita* and the presence of *M. javanica* and *M. arenaria*, either as single or mixed populations, are in agreement with reports for local maize production areas (Riekert, 1996; Riekert & Henshaw, 1988; Mc Donald *et al*., 2017; SAPPNS[1]). A recent study by Agenbag (2016) also recorded the predominance of *M. incognita* and *M. javanica* in maize roots, with *M. arenaria* occurring to a lesser extent. During this study, these two species were found in areas where different crop rotation programmes are practiced, e.g. grasses, maize, oat (*Avena sativa* L.), potato, soybean, sugar bean (*Phaseolus vulgaris* L.), sunflower and wheat. All such crops have been reported as susceptible hosts to *M. incognita* and *M. javanica* (Kleynhans, 1991; Kleynhans *et al.*, 1996; Fourie *et al.*, 2001; Mc Donald *et al*., 2017) and will contribute towards increases in their population densities. This is an important aspect that needs to be addressed by means of host status assessments of cultivars of the different crops towards the target *Meloidogyne* spp.

This way, resistant or poor hosts of the various crops can be recommended for inclusion in cropping cycles to contribute towards lowering *Meloidogyne* spp. population densities in local agricultural soil. A novel finding, however, is for *M. enterolobii* that parasitised maize sampled from a rain-fed field (87 % sand content) in the Bethal area where minimum tillage was practiced. This species occurred in a mixed population with *M. incognita*, with which can be if all morphological and morphometrical are not taken into account. *Meloidogyne enterolobii* was widespread in this field and were present in 88 % of the samples obtained, and had a fairly high PV and MPD (156 and 166, eggs and J2 / 50 g roots, respectively). More important is that a soybean-maize cropping sequence is used by this producer, with soybean being listed as a susceptible host of *M. enterolobii* (Anonymous, 2014). By contrast, maize is listed as a non-host for this species (Anonymous, 2014). Interestingly, *M. enterolobii* was also recorded in 2016 from the Delmas area situated approximately 100 km from Bethal but from roots from potato (Agenbag, 2016). Hence in this maize production area, crops susceptible to *M. enterolobii* (EPPO, 2014; Onkendi & Moleleki, 2013) are grown that will support the development and reproduction of *M. enterolobii*. This species has also been identified from potato tubers grown in KwaZulu Natal (Onkendi & Moleleki, 2013) in maize-based cropping systems. Since *M. enterolobii* is reported to be more aggressive than its thermophilic counterparts (Anonymous, 2014), knowledge about its distribution in local crop production areas is crucial. Only when such information becomes available, timely and effective nematode control strategies can be employed to prevent *M. enterolobii* from reaching unmanageable population densities.

Except for *Meloidogyne*, valuable information has also been generated about the abundance and occurrence of *Pratylenchus* in local maize fields as a result of this study. The presence of *Pratylenchus* individuals in 99 % of the 5-g root samples obtained during this survey confirm that this genus is an economically important nematode pest of maize in South Africa (De Waele & Jordaan, 1988; Mc Donald *et al*., 2017). More recent diagnostic information by Bekker *et al*. (2007) andFourie *et al*. (2011), as well as research data by Bekker (2016) is also in agreement with results from this study that *Pratylenchus* is abundant in local maize production areas. The highest PV and MPD for *Pratylenchus* (899 and 895, respectively) were for a no-tilled, rain-fed maize field in the Sannieshof area (North West Province) where sunflower is rotated with maize in soils with a 78 % sand content. Forty-nine percent of the fields had lower PVs and MPDs for *Pratylenchus* of between 100 and 500 individuals / 5 g roots, while 50 % had PVs and MPDs of <100 individuals / 5 g roots. No damage threshold values and/or yield loss data exist for *Pratylenchus* under local environmental conditions for maize.

Results generated during this study hence showed that priority should be given for research to be done on lesion nematodes and maize. This genus has a wide host range (Bridge & Starr, 2007), infecting a wide range other grain (Mc Donald *et al*., 2017), leguminous (Fourie *et al*., 2001), vegetable (Jones *et al*., 2017) and industrial crops (Van Biljon, 2017) used in local maize-based cropping systems. High population build-ups of this genus can thus be experienced should proper nematode management strategies not be applied. To implement such strategies, knowledge about the identity of *Pratylenchus* spp. is, however, crucial. No species identification was done for lesion nematodes for this study since the emphasis was on *Meloidogyne*. However, a follow-up survey will be conducted in the 2018 summer-growing season at the same localities during which species identification will be done.

Another interesting finding was that the presence of high *Rotylenchulus* eggs and juveniles in maize roots from fields under both conventional and conservation practices reported during a recent study (Bekker *et al*., 2016), was not found during this study. *Rotylenchulus* juveniles was, however, present in low numbers in soil samples, agreeing that this genus is omnipresent in local maize (Keetch & Buckly, 1984; De Waele & Jordaan, 1988; Kleynhans *et al*., 1996, Marais & Swart, 2007; SAPPNS), soybean (*Glycine max* L. Merr) (Fourie *et al*., 2001), wheat (*Triticum aestivum* L.) (Jordaan *et al*., 1992) and sunflower (*Helianthus annuus* L.) fields (Bolton *et al*., 1989).

Except for *Meloidogyne* and *Pratylenchus* that also dominated in soil samples, six other plant-parasitic genera, namely *Helicotylenchus* Steiner, 1945; *Rotylenchulus* Linford & Oliveira, 1940; *Nanidorus* Siddiqi, 1974 and/or *Tylenchorhynchus* Cobb, 1913 and *Xiphinema* Cobb, 1913 were identified (in order of predominance). In addition, individuals belonging to the genus *Criconema* were also identified. Except for *Meloidogyne*, no species identification was attempted for any of these genera. Species of these genera have, however, been listed in association with local maize crops (Kleynhans *et al.*, 1996; Riekert, 1996; Riekert & Henshaw, 1998; Fourie *et al.*, 2001; Bekker, 2016; Mc Donald *et al*., 2017; SAPPNS[1]) but their damage potential to the crop remains unknown. This is mainly because these nematode genera often occur sporadic and in low population densities in local maize production areas. Nonetheless, their presence in maize fields should not be ignored or research on their presence and influence neglected..

2.7 References

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Chapter 3: Population development of *Meloidogyne* spp. in a susceptible maize cultivar and the effect of a seed treatment on the reproduction of this nematode genus

3.1. Introduction

Maize producers in South Africa experience root-knot nematodes (*Meloidogyne* spp.) as one of the major pests that damage their crops. *Meloidogyne incognita* (Kofoid & White, 1919) Chitwood, 1949 and *Meloidogyne javanica* (Treub, 1885) Chitwood, 1949, in particular are the predominant species that are present in soils of local production areas (Mc Donald *et al*., 2017). *Meloidogyne arenaria* (Neal, 1889) Chitwood, 1949, is also present in local maize fields but to a lesser extent than the afore-mentioned species (Kleynhans, 1991; Kleynhans *et al*., 1996; Marais, 2016; Agenbag, 2016; SAPPNS[1]). These three *Meloidogyne* spp. occur either as single- and/or mixed species populations in local maize (*Zea mays* L.) production areas (Agenbag, 2016).

The mere presence of plant-parasitic nematodes in crop fields is not a guarantee that maize production will be adversely affected. Crop damage will only occur when the target nematode pestpopulation exceeds the damage threshold level (Brown, 1987; Schomaker & Been, 2006; Khan, 2008). Moreover, various factors contribute towards crop damage and/or yield reduction caused by nematode pest infections. These, for example, include environmental conditions (rainfall and temperature in particular), cultivar grown, soil type, cropping history, the nematode species and/or race present as well as distribution and the reproduction potential of the target nematode pest (Schomaker & Been, 2006; Khan, 2008).

Literature about the relationships between yield loss of maize and initial population densities (Pi) of plant-parasitic nematodes, particularly *Meloidogyne*, is scarce. A few studies only have been published on this topic for *M*. *incognita* and *M javanica* (Di Vitto *et al*., 1980; Fourie *et al*., 2008). In a South African study, strong non-linear relationships between Pi (0 to 20 000 eggs and J2 per seedling) and final population densities (Pf) of *M. incognita* and *M. javanica* were reported for resistant and susceptible maize genotypes (Ngobeni *et al*., 2010). Yield losses for the susceptible standards at Pi level of 20 000 eggs and J2/seedling were 55 and 72 % for*M. javanica* and *M. incognita*, respectively. From Italy, Di Vito *et al*. (1980) reported that an inverse relationship between Pi and Pf population densities for a maize hybrid. In their study, the lowest Pi of 0.5 eggs of *M. incognita* per gram soil at planting resulted in a 458-times increase in Pf at harvest. Moreover, a strong non-linear relationship

existed between Pi and maize yield, with a damage threshold level of 10 eggs per gram of soil being recorded. Ultimately, growth reduction of inoculated maize plants was 90 % compared to that for non-inoculated (control) plants.

Maize yield losses, ranging between 10 and 60 %, have been attributed to *Meloidogyne* spp. parasitism in local maize crops (Keetch, 1989; Riekert & Henshaw, 1998). A large percentage of local maize crops are produced on sandy soils, with a clay content of less than 10 % (Du Plessis, 2003). Root-knot nematodes optimally develop and reproduce in such sandy soils (Kim *et al.*, 2017) and it is hence no surprise that a large percentage of local producers experience severe problems with these pests. The root-knot nematode problem in local maize fields is further aggravated by the withdrawal of highly effective synthetic nematicides, e.g. aldicarb and endosulfan (Haydock *et al.*, 2013; Senwes, 2012; Verdoorn, 2012). Growing concerns about the pollution of natural resources (ground water, soil and air) as well as poisoning of animals and humans resulting from the use of Class I, Red-Band nematicides furthermore expedited their withdrawal from world markets. Therefore, two new-generation seed treatment products have been registered during the past five years on maize in South Africa to protect crops against nematode pests (Van Zyl, 2013; Bayer CropScience, 2014). One of these products is Avicta® 500FS, of which the active substance is abamectin: a macrocyclic lactone derived from the soil bacterium *Streptomyces avermitilis* that has nematicidal properties (Putter *et al*., 1981). This product has a different mode of action (Turner & Schaeffer, 1989) than that of synthetically-derived nematicides that are currently available and has been used successfully to reduce population densities of various plant-parasitic nematodes, including *Meloidogyne* spp., in a range of crops (Syngenta, 2017).

The use of seed treatments is an attractive alternative for nematode management since it requires less chemical inputs than large scale field applications of nematicides and it reduces the negative impact of chemicals on animals, humans and the environment (Cabrera *et al*., 2009). However, controversy exists among South African maize producers about the efficacy of seed treatments with nematicidal actions since they are not regarded as effective as the synthetically-derived products. This is especially the case where high *Meloidogyne* spp. population densities occur (Personal communication, Prof Driekie Fourie, NWU, 6 November 2017).

The main aim of quantitative Nematology research is to establish the relationship between pre-plant population densities of the target plant-parasitic nematode species and their effect on crop performance (e.g. plantgrowth, yield and/or quality) (Barker & Olthof, 1976; Schomaker & Been, 2006). To protect crops against nematode pests, quantitative knowledge of the relationships between the target nematode pest before/during planting (initial inoculation densities) versus later in the growing season (e.g. just before harvesting) should be obtained. Damage threshold values are thus needed to optimize nematode pest management programs (Ferris, 1978; Schomaker & Been, 2006). Such information for *Meloidogyne* spp. and maize is crucial for local maize producersto enable them to make informed decisions about which management strategy(s) to use. The need, therefore existed to quantify i) the relationship between the Pi and Pf of single and mixed populations of *M. incognita* and *M. javanica* in roots of a local susceptible maize genotype and ii), the effect of a registered seed treatment on population development of *Meloidogyne* spp.

3.2. Materials and methods

3.2.1 Micro plot studies

3.2.1.1 Study 1

3.2.1.1.1. Set up and soil preparation

This study was conducted in micro plots that are situated on the premises of the Agricultural Research Council - Grain Crops Institute, Potchefstroom (26° 43’ 0” S; 27° 6’ 0” E), North West Province, South Africa. The micro plot system is installed over a drainage system and consists of fiberglass tubes (1 m in diameter and 1 m deep) that are covered with hail nets to protect crops against hail. During the summer-growing season of 2013/14,seeds of the conventional, root-knot nematode susceptible maize cultivar DCK8010 was planted in sandy soil in the micro-plots. This cultivar was used for all experiments in this study since it is susceptible to both *M. incognita* and *M. javanica* (Ngobeni *et al.*, 2010). Also as seeds of cultivars are treated with combined products to protect maize against diseases (fungi), pests (nematodes and insects) and weeds, non-treated seed of this cultivar only could be obtained at the time this study commenced.

The soil was collected from a maize field in the Leeudoringstad area (North West Province) and consisted of 6 % clay, 0 % silt, 94 % sand, 0.25 % organic carbon (C) with a pH (H_2O) of 6.37. Three weeks before planting, the soil in each micro plot was fumigated with Telone® II (active substance: 1,3 dichloropropene @ 1 110g / l) at a dosage rate of 150 l/ha. Before

planting, the soil in each microplot tube was loosened to allow air flow between soil particles. Fertiliser was applied in each microplot according to soil analyses and comprised of 10 g KAN at planting. Also, 10 g KAN was applied 30 days after emergence as a top dressing. At planting, furrows were made in each half of each micro plot tube. Inter-row and intra-row spacing of maize seeds were 30 cm and 40 cm, respectively. Two separate experiments for each of the two single-species populations of *M. incognita* and *M. javanica* were conducted concurrently.

3.2.1.1.2 Root-knot nematode inoculum (origin and *in vivo* rearing)

The two root-knot nematode species, *M. incognita* and *M. javanica*, used as inoculum for this study originated as single species populations from maize roots from the Vryburg area and pumpkin roots from the Marble Hall area, respectively. The identity of these *M. incognita* and *M. javanica* populations was elucidated using perineal-pattern morphology (Kleynhans, 1991) and the molecular sequence-derived amplified region – polymerase chain reaction (SCAR-PCR) method (Zijlstra *et al*., 2000). Single egg masses from each of the populations were then inoculated on roots of susceptible tomato seedlings (cultivar Floradade) and reared in separate glasshouses at the premises of the Nematology Laboratory of the North-West University. Fifty-six days after rearing of the respective *Meloidogyne* spp. commenced, infected roots of tomato plants of each species were removed from the pots they were grown in. The infected roots for each species were kept separate and rinsed with tap water to remove all excessive soil and debris. The roots were then cut into approximately 1-cm pieces and eggs and J2 of each species extracted separately using the modified sodium hypochlorite (NaOCl) method of Riekert (1995) (see Chapter 2, Paragraph 2.2.2.1.2)

Eight treatments (Pi = 0, 100, 500, 1 000, 2 500, 5 000, 7 500 and 10 000) eggs and second-stage juveniles (J2) of each of the two respective root-knot nematode species were prepared and inoculated per maize seed. Six maize seeds of the root-knot nematode susceptible cultivar DKC8010 were placed approximately 5 cm deep in a furrow made in each half of each micro plot tube. After nematode inoculation, the furrows were closed and 15 mm irrigation (to allow nematode survival) applied using a sprinkle irrigation system. The same amount of irrigation was applied when the top soil dried until germination of the seeds took place. Thereafter, approximately 25 mm of irrigation was applied three times a week except when it rained. Weeds were controlled manually and no diseases and pests were observed during the duration of the experiments. The experimental layouts of the two experiments,

one for *M. incognita* and one for *M. javanica*, were randomized complete, split plot designs, with five replicates per Pi level.

3.2.1.1.3 Nematode sampling, extraction and reproduction assessment

Fifty-six days after planting and nematode inoculation, maize plants of cultivar DKC8010 were removed from each treatment (Pi level) and roots excised from the aerial parts. The entire root system of each plant were then rinsed with a gentle stream of tap water to remove excess soil and debris, weighed and cut into approximately 1-cm pieces. The entire root system of each plant were used since reproduction factors (Rfs) were calculated to determine the reproduction potential of the respective *Meloidogyne* spp. for each Pi. Eggs and J2 of each species were extracted from the roots using the modified NaOCl method (Riekert, 1995) as described in Chapter 2, Paragraph 2.2.2.1.2. Final population levels of the mixed *Meloidogyne* spp. for each plant's roots were determined in a De Grisse counting dish (De Grisse, 1963) using a stereo microscope (40× magnification). The following nematode parameters were finally determined:

i) egg and J2 numbers / root system,

ii) reproduction factor (Rf) value (Rf) using the equation of Oostenbrink: Rf = Pf / Pi as published by Windham & Williams (1987).

3.2.1.2 Study 2

The same procedure for a second micro plot study was generally followed as explained in Paragraphs 3.2.1.1.1 to 3.2.1.1.3, except that a mixed *Meloidogyne* spp. population was used. Also, one treatment constituted seed of cultivar DKC8010 coated with Avicta® 500FS at the registered dosage rate of 0.5 mg active substance / seed (Van Zyl, 2013). Seed treatment was done by personnel of Syngenta South Africa, the owner company of this registered nematicidal product (Van Zyl, 2013; Anonymous, 2017). The other treatment constituted of non-treated seed anpd represented the control. The Pi levels for this study were different from those for icro Plot Study 1 and represented 0, 50, 100, 500, 1 000 and 5 000, with six replicates for each Pi level. This experiment was done during the summer growing season of 2013/14 in a similar micro plot setup situated on the premises of the ARC – GCI as referred to in Paragraph 3.2.1.1.1. The same nematode parameters were used as mentioned in Paragraph 3.2.1.1.3. This study was also repeated in a glasshouse as described in Paragraph 3.2.1.3.

3.2.1.3 Glasshouse study

3.2.1.3.1 Root-knot nematode inoculum (origin and *in vivo* rearing)

A mixed population of *M*. *incognita*and *M. javanica* (70:30 ratio) was obtained from infected maize roots that were cultivated in the Vryburg region in the Northern Cape Province. This population was reared in a glasshouse in a 25 000 cm^3 plastic pot *on Meloidogyne* susceptible tomato cultivar Rodade (*Lycopersicon esculentum* L.). The *in vivo* rearing of this *Meloidogyne* population was done at the Eco-Rehab Plant Protection Unit, North-West University in Potchefstroom according to the procedure described by Fourie *et al*. (2012). Eggs and second-stage juveniles (J2) of this population were extracted from the infected tomato roots 56 days after rearing commenced using the adapted NaOCl method (Riekert, 1995).

3.2.1.3.2 Trial set up and nematode inoculation

Sandy loam soil (5 % clay, 94 % sand, 1 % silt and 0.47 % organic matter content) with a pH (H_2O) of 7.2 were used to fill 4 000 cm^3 plastic pots. The soil was fumigated with Telone® II (active substance: 1,3dichloropropene @ 1 110 g / l at a dosage rate of 150 l / ha) four weeks before the experiment commenced. Seeds coated with Avicta® 500FS (Anonymous, 2017) and those not treated with this product (control) represented the two treatments and were obtained from Mr. Lukas Meyer of Syngenta. The conventional cultivar used was DKC8010 that is commercially available for local producers. Six treatments, representing Pi = 0, 50, 100, 500, 1 000 and 5 000 eggs and J2 were prepared from the inoculum source (see Paragraph 3.2.1.3.1) for each of the two treatments and inoculated in 1 ml of tap water at the bottom of each 5-cm deep hole in each pot. Each pot was placed in its own saucer/tray. One maize seed was next placed into the hole and the hole covered with soil. One-hundred ml of tap water was carefully added to the soil surface of each pot every second day to keep the nematodes alive and allow germination of the seed. After the seeds germinated, the pots were watered with 500 ml of tap water three times a week for the first 30 days and thereafter with 1 l of tap water three times a week until the trial was terminated at 56 days after planting. A soluble fertiliser mixture, Nutrifeed (https://www.starkeayres.co.za/) was used of which 10 g was dissolved in 5 l of tap water and added to the seedlings seven days after planting and again 30 days after planting. The trail layout was a randomized complete split-plot design, consisting of six replicates each for both the Avicta-treated and non-treated treatments.

3.2.1.3.3 Nematode sampling, extraction and reproduction assessment

Fifty-six days after planting and nematode inoculation, the maize plants were removed from pots and their roots excised from the aerial parts according to the same procedure as described for the micro plot studies in Paragraph 3.2.1.1.3. The same nematode parameters used for the micro plot studies (see Paragraph 3.2.1.1.3) were also determined.

3.2.2 Data analyses

Meloidogyne egg and J2 data per root system obtained for the micro plot and glasshouse studies (dependent variables) were regressed non-linearly on the various Pi levels (independent variable) using polynomial($y = a*x^2 + b*x + c$) and logarithmic ($y = a*x + b$) models. These functions are representative of the data ranges obtained for the respective experiments. Nematode (egg and J2 numbers per root system), both real and log(x+1) transformed numbers for the micro plot and glasshouse studies, were also submitted to factorial Analysis of Variance (ANOVA). The treatments represented the main effects and Pi levels the subfactor. Means were separated by the Tukey Test where $P \leq 0.05$. All statistical analyses were done using Statistica Version 13.2 (www.statsoft.com).

3.3 Results

3.3.1. Microplot Study 1

3.3.1.1 *Meloidogyne* egg and J2 numbers / root system

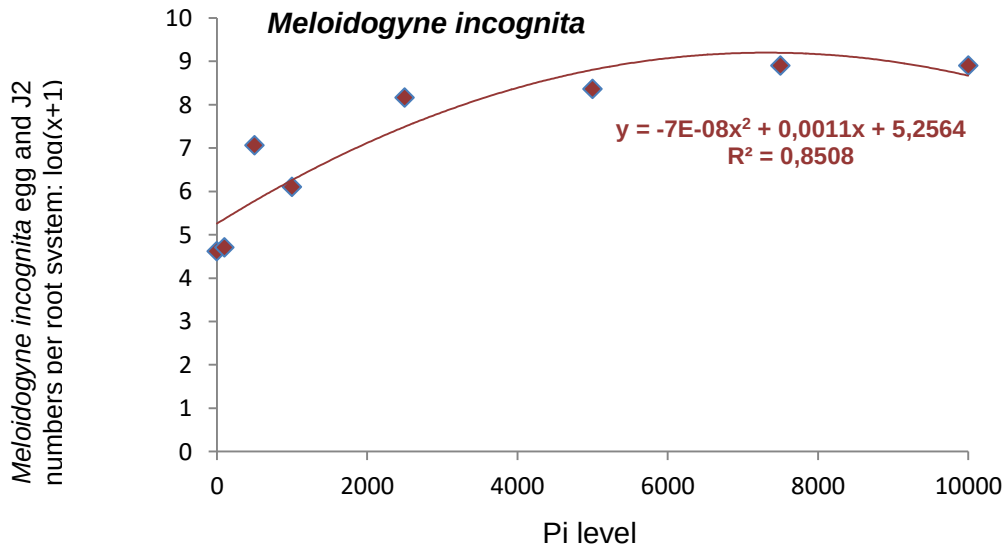

Figure 3.1A Log (x+1) transformed data indicating the effect of initial population densities of a *Meloidogyne incognita* on its finalpopulation densities in roots of maize cultivar DCK8010.

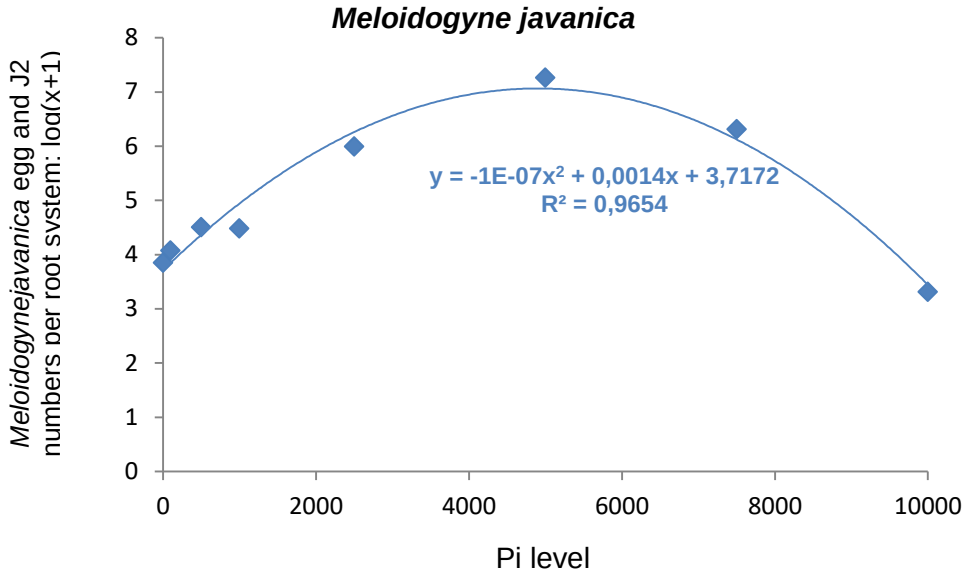

Figure 3.1B Log (x+1) transformed data indicating the effect of initial population densities of a *Meloidogyne javanica* on its final population densities in roots of maize cultivar DCK8010.

Table 3.1 Final population densities of *Meloidogyne incognita* and *Meloidogyne javanica* at different initial inoculation densities in roots of a susceptible maize cultivar (DCK8010).

Pi level	**Pf in roots: *M. incognita***	**Pf in roots: *M. javanica***
0	4.6* (952** ±752.9***) bAB	4.1* (324** ±264.0***) aAB
100	4.6 (567 ±378.8) bAB	4.0 (238 ±156.4) aA
500	6.9 (2 149 ±1 634.8) abABC	4.5 (308 ±166.2) aAB
1 000	6.1 (1 092 ±789.1) abABC	4.4 (182 ±129.0) aAB
2 500	8.0 (3 724 ±939.8) aABC	5.9 (700 ±443.5) aABC
5 000	8.2 (4 956 ±1 606.7) aBC	7.3 (1627 ±700.4) aABC
7 500	8.9 (8 855 ±2 505.3) aC	6.2 (770 ±285.4) aABC
10 000	8.8 (8 113 ±3 129.6) aC	6.4 (1 036 ±496.6) aABC
P value	0.000	0.40
F ratio	6.931	2.523

Table 3.1 Continues

Interaction data: *M. incognita* vs *M. javanica*
P value = 0.801
F ratio = 0.54

*Log (x+1) transformed data; **Real means; ***Standard Error; means in the same column followed by the same lower case letter do not differ significantly ($P \leq 0.05$) according to Tukey's HSD Test, while means in the same row followed by the same uppercase letter do not differ significantly ($P \leq 0.05$) according to Tukey's HSD Test.

The non-linear, polynomial relationships between Pi and Pf for both *M. incognita* and *M. javanica* egg and J2 numbers per root system were strong and highly significant (Figs. 3.1A & B). These models explained 85 and 96 % of the variation for Pf for *M. incognita* and *M. javanica*, respectively. The regression line for *M. javanica* started leveling off from Pi level 5 000 eggs and J2 / root system, while a similar trend was evident for *M. incognita* but only at Pi of 7 500 eggs and J2 / root system.

No significant no interaction existed between the two species regarding Pf values obtained for the different Pi levels (Table 3.1). However, for *M. incognita* significantly ($P \leq 0.05$) higher egg and J2 population densities were recorded from Pi levels 2 500 onwards compared to Pi levels 0 to 1 000. For *M. javanica*, however, no significant ($P \leq 0.05$) differences were recorded among the eight Pi levels.

3.3.1.2 Root mass

Table 3.2 Final root masses of *Meloidogyne incognita* and *Meloidogyne javanica* infected roots of a susceptible maize cultivar (DCK8010).

Pi level	**Root mass (g): *M. incognita* infected plants:**	**Root mass (g): *M. javanica* infected plants**
0	50.0* ±0***Aa	45*±9.1***aA
100	46±4.14aA	48±1.6aA
500	44±3.5aA	48±1.6aA
1 000	49±1.5aA	46±3.6aA
2 500	45±3.7aA	47±3.3aA

Table 3.2 Continues

5 000	43±5.4aA	40±8.6aA
7 500	48±1.7aA	46±2.7aA
10 000	46±3.9aA	44±4.6aA
P value	0.811	0.397
F ratio	0.522	1.092
Interaction data: Root mass of *M. incognita*vsRoot mass of *M. javanica*		
P value = 0.872		
F ratio = 0.441		

*Real means; **Standard Error; means in the same column followed by the same lower case letter do not differ significantly ($P \leq 0.05$) according to Tukey's HSD Test, while means in the same row followed by the same uppercase letter do not differ significantly ($P \leq 0.05$) according to Tukey's HSD Test.

No significant interaction was evident between the two *Meloidogyne* spp. regarding the root mass of plants for the different Pi levels (Table 3.2). No significant differences were also observed for the individual species regarding root mass data for the different Pi levels.

3.3.2. Microplot Study 2

3.3.2.1 *Meloidogyne* egg and J2 numbers / root system

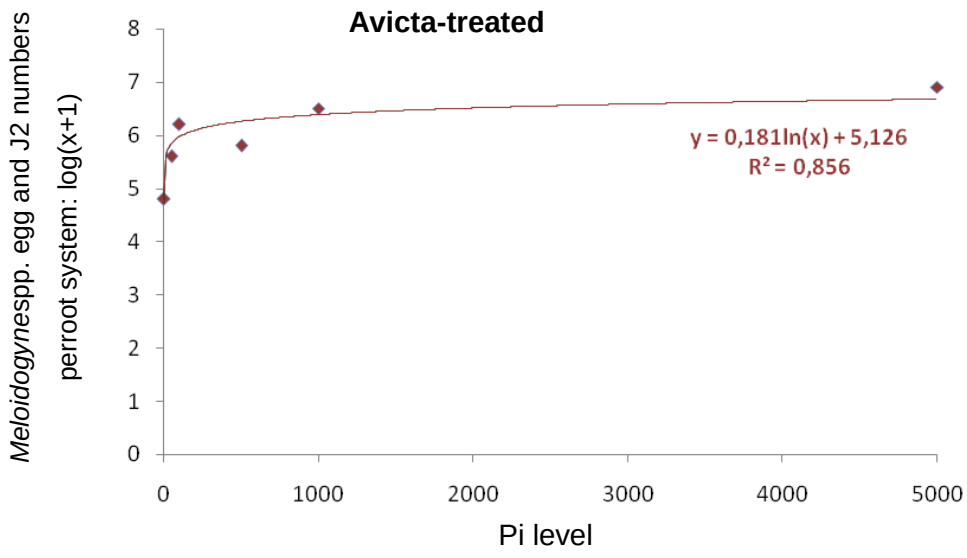

Figure 3.2A Log (x+1) transformed data indicating the effect of initial population densities of a mixed *Meloidogyne incognita* and *Meloidogyne javanica* (70:30 ratio) on their reproduction potential in roots of maize cultivar DKC8010 of which seed was treated with a registered nematicide seed treatment.

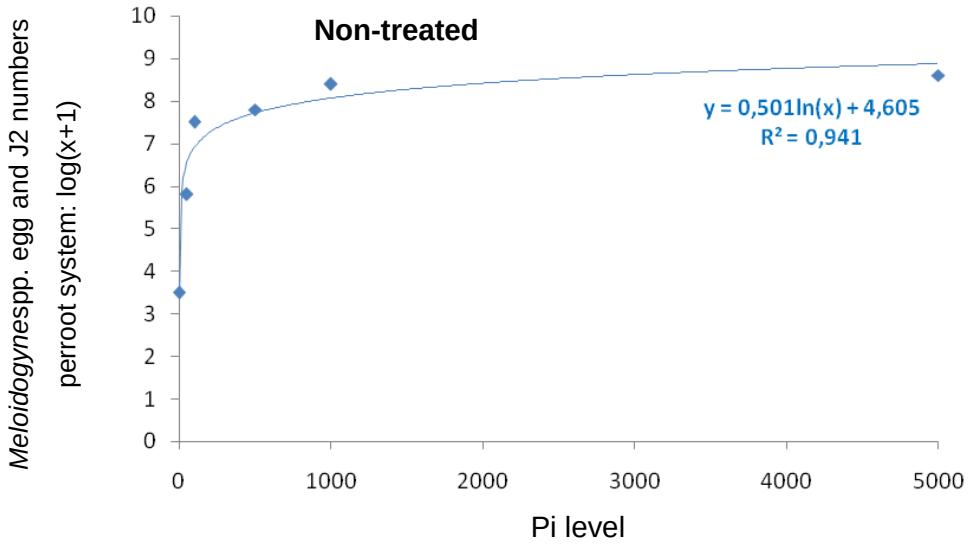

Figure 3.2B Log (x+1) transformed data indicating the effect of initial population densities of a mixed *Meloidogyne incognita* and *Meloidogyne javanica* (70:30 ratio) on their reproduction potential in roots of maize cultivar DKC8010 of which seed was not treated with a nematicide product.

Table 3.3 Final population densities of a mixed *Meloidogyne incognita* and *Meloidogyne javanica* population for Avicta® 500FS treated and non-treated seeds at different initial inoculation densities in roots of a susceptible maize cultivar DKC8010.

Pi level	**Pf in roots:** **Avicta-Treated**	**Pf in roots:** **Non-treated**
0	4.8* (128** ±22.8***)aBF	3.5* (99** ±58.0***)bF
50	5.6 (394 ±133.9)abABF	5.8 (370 ±73.4)cABC
100	6.2 (713 ±274.5)abABC	7.5 (2 082 ±603.2)aACDE
500	5.8 (455 ±166.7)abAB	7.8 (2 491 ±334.4)aCDE
1 000	6.5 (1 225 ±433.6)abABCD	8.4 (4 668 ±525.4)aDE

Table 3.3 Continues

5 000	6.9 (1 801 ±893.6)bACDE	8.6 (5 409 ±835.2)aE
P value	0.020	0.000
F ratio	3.313	23.86
Interaction data: Non-treated vs treated seeds		
P value = 0.002		
F ratio = 4.43		

*Log (x+1) transformed data; **Real means; ***Standard Error; means in the same column followed by the same lower case letter do not differ significantly ($P \leq 0.05$) according to Tukey's HSD Test, while means in the same row followed by the same uppercase letter do not differ significantly ($P \leq 0.05$) according to Tukey's HSD Test.

The non-linear, logarithmic relationships between Pi and Pf for both the Avicta- as and non-treated seeds for a mixed *M. incognita* and *M. javanica* population per root system were strong and highly significant, with 86 and 94 of the variance respectively being explained for the two treatments (Figs. 3.2A & B). According to the regression lines for both treatments, no levelling off were evident since Pf increased with increasing Pi levels.

A significant ($P \leq 0.05$) interaction existed between the two treatments regarding the Pf values obtained for the *Meloidogyne* spp. at the different Pi levels (Table 3.3). For the Avicta treatment, Pi level 10 000 had a significantly ($P \leq 0.05$) higher Pf than Pi level zero while the other Pi levels did not differ from either these two Pi levels. For the non-treated control, Pi levels 500 to 10 000 had significantly ($P \leq 0.05$) higher Pf levels of the *Meloidogyne* spp. compared to Pi levels zero and 100. The latter two Pi levels also differed ($P \leq 0.05$) significantly from each other for Pf.

3.3.2.2 Root mass

Table 3.4 Final root masses of a mixed *Meloidogyne incognita* and *Meloidogyne javanica* population for Avicta® 500FS treated and non-treated seeds at different initial inoculation densities in roots of a susceptible maize cultivar DKC8010.

Pi level	**Pf in roots: Avicta-Treated**	**Pf in roots: Non-treated seeds**
0	42* ±5.4**aA	48* ±6.0**aA
50	47±5.1aA	47 ±5.0aA
100	35±6.6aA	43±6.1aA
500	39±6.7aA	50 ±4.5aA
1 000	43±5.0aA	48 ±4.6aA
5 000	42±5.6aA	50 ±4.5aA
P value	0.445	0.337
F ratio	0.987	1.206
Interaction data: Root mass of *M. incognita*vs Root mass of *M. javanica*		
P value = 0.745		
F ratio = 0.540		

*Real means; **Standard Error; means in the same column followed by the same lower case letter do not differ significantly ($P \leq 0.05$) according to Tukey's HSD Test, while means in the same row followed by the same uppercase letter do not differ significantly ($P \leq 0.05$) according to Tukey's HSD Test.

No significant interaction was evident between the two treatments regarding the root mass of plants infected with the different Pi levels of the *Meloidogyne* spp. (Table 3.4). No significant differences were also observed for the individual species regarding root mass data for the different Pi levels.

3.3.3 Glasshouse study

3.3.3.1 *Meloidogyne* egg and J2 numbers / root system

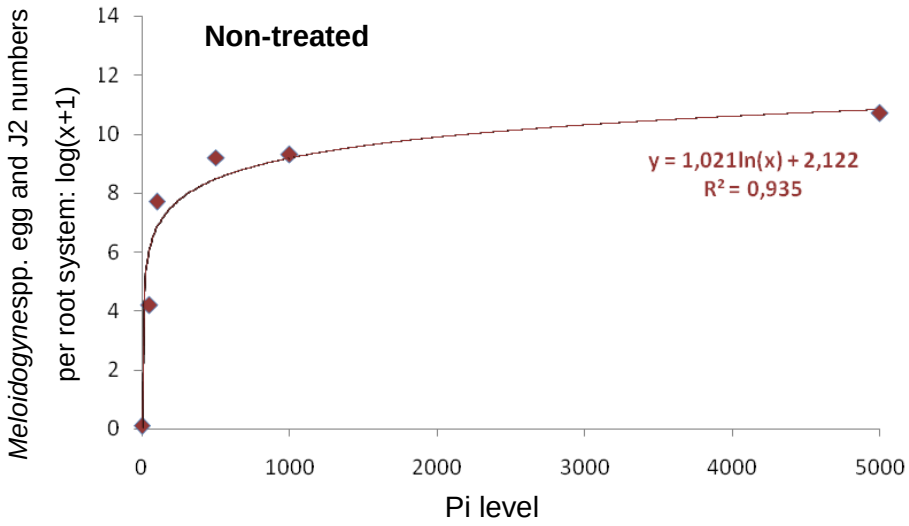

Figure 3.3A Log (x+1) transformed data indicating the effect of initial population densities of a mixed *Meloidogyne incognita* and *Meloidogyne javanica* (70:30 ratio) on their reproduction potential in roots of maize cultivar DKC8010 of which seed was not treated (control) with a nematicide seed treatment.

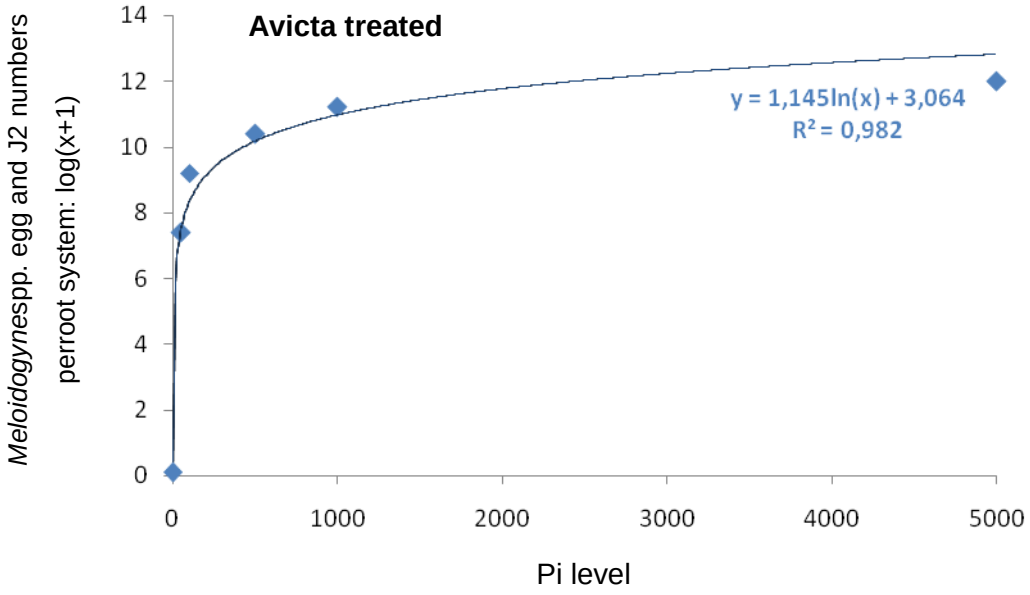

Figure 3.3B Log (x+1) transformed data indicating the effect of initial population densities of a mixed *Meloidogyne incognita* and *Meloidogyne javanica* (70:30 ratio) on their reproduction potential in roots of maize cultivar DKC8010 of which seed was treated with a registered nematicidal seed treatment.

Table 3.5 Final population densities of a mixed *Meloidogyne* spp. population, at different initial inoculation densities, in roots of a susceptible maize cultivar (DKC8010) of which the seeds were either treated with Avicta® 500FS or not (control)

Pi level	Pf in roots: Non-treated	Pf in roots: Avicta treated
0	0* (0** ±0***)cE	0* (0** ±0***)cE
50	7.4 (1 989 ±577.7)dB	4.2 (463 ±236.5)dF
100	9.2 (19 018 ±9 149.6)eABC	7.7 (3 278 ±911.2)aBC
500	10.4 (48 854 ±18 503.9)aACD	9.2 (10 407 ±1 527.0)abABC
1 000	11.2 (90 405 ±31 020.8) abAD	9.3 (12 408 ±3 476.4)abABC
5 000	12.0 (158 953 ±19 426.5) bAD	10.7(72 310 ±18 977.2)bD
P value	0.001	0.001
F ratio	242.8	38.28
Interaction data: Non-treated vs treated seeds		
P value = 0.071		
F ratio = 2.2		

*Log (x+1) transformed data; **Real means; ***Standard Error; means in the same column followed by the same lower case letter do not differ significantly ($P \leq 0.05$) according to Tukey's HSD Test, while means in the same row followed by the same uppercase letter do not differ significantly ($P \leq 0.05$) according to Tukey's HSD Test.

The non-linear, logarithmic relationships between Pi and Pf for both the Avicta- and non-treated seeds for a mixed *M. incognita* and *M. javanica* population per root system were strong and highly significant, with 94 and 98 % of the variance, respectively, being explained for the two treatments (Figs. 3.2A & B). According to the regression lines for both treatments, no levelling off were evident since Pf increased with increasing Pi levels.

No significant interaction existed between the two treatments regarding thePf values obtained for the *Meloidogyne* spp. at the different Pi levels (Table 3.3). For the Avicta treatment, Pi level 10 000 had a significantly ($P \leq 0.05$) higher Pf than Pi level zero while the other Pi levels did not differ from either these two Pi levels. For the non-treated control, Pi

levels 500 to 10 000 had significantly ($P \leq 0.05$) higher Pf levels of the *Meloidogyne* spp. compared to Pi levels zero and 100. The latter two Pi levels also differed ($P \leq 0.05$) significantly from each other for Pf.

3.3.3.2. Plant data (Root and aerial mass)

Replicates for both treatments for root and aerial mass differed significantly from each other and therefore warrants no further discussion.

3.4. Discussion

The very high probability and significance values obtained between Pi and Pf of *Meloidogyne* spp. for susceptible maize cv. DKC8010 for both micro plot (semi-controlled conditions) and glasshouse (controlled conditions) studies indicated the high repeatability of the results. The pronounced effect of Avicta® 500FS in terms of reducing *Meloidgyne* spp. Pfs is also a positive result. The lack of yield and/or plant growth data is, however, disappointing and will be obtained in future research. Only when such data are added can a complete picture of the effect of differential Pi of the economically important *Meloidogyne* spp. on maize yield be obtained. Optimising yield is the ultimate goal of farmers to ensure a higher income.

The substantial difference in population development of *M. incognita* and *M. javanica* in roots of the susceptible maize (cultivar DKC8010) used in micro plot study 1 is interesting, but not unexpected. It is known that the reproduction potential of different *Meloidogyne* spp. populations that are geographically isolated from one another differs (Anwar *et al*., 2000). This phenomenon has also been recorded in host status screenings of maize genotypes adapted to South African environmental conditions to these two *Meloidogyne* spp. in concurrent experiments (Ngobeni *et al*., 2010). The reproduction potential of the *M. incognita* population used in this study was substantially higher than that of the *M. javanica* population . This manifested in *M. incognita* population levels, at the highest Pi of 10 000 eggs and J2 / root system, being 7.8 times higher than that for its counterpart species. This finding may offer an explanation why *M. incognita* is listed as the predominant root-knot nematode species in local maize production areas (Riekert, 1996; Riekert & Henshaw, 1998; Mc Donald *et al*., 2017), which was confirmed by the extensive survey done as part of this study (see Chapter 2, Paragraph 2.5.2). Another interesting finding is that Pf levels started leveling off for *M. incognita* in this micro plot study from Pi 7 500, which is substantially higher than Pi

1 000 reported by Fourie *et al*. (2009) for the conventional, susceptible cultivar AFG4410. However, for *M. javanica the* Pf levels for the latter two studies were in a similar range, being Pi 5 000 for this study and 7 500 for the study of Fourie *et al*. (2009). Differences experienced for Pi between these studies might, however, be attributed to the different *M. incognita* populations, as well as the different maize cultivars used. It is known that differences in the reproduction potential exists between *Meloidogyne* spp. (Anwar *et al*., 2000) and populations of the same species (Hussey & Janssen, 2002). Furthermore, the host status of maize cultivars to *Meloidogyne* spp. has also been shown to differ (Ngobeni *et al*., 2010). The final population density per root system of *M. incognita* in roots of conventional cultivar AFG4410 , when inoculated with 10 000 eggs and J2 per seedling, was recorded as 16 640 eggs and J2, while that for DKC8010 has been recorded as 19 079 (Ngobeni *et al*., 2010). A similar scenario existed for *M. javanica* regarding the susceptible cultivars used in different studies (Ngobeni *et al*., 2010). Hence the different *M. incognita* populations used, differences in the host status of the cultivars used as well as differences in glasshouse conditions may have contributed two different results for these two studies. Ultimately, no concrete conclusion can be made in this regard since additional research is need to elucidate this scenario.

Also noteworthy is the sharp decline in reproduction potential of *M. javanica* compared to that of *M. incognita* in this micro plot study at Pi levels 7 500 and 10 000 eggs and J2 / root system. This phenomenon can also not be explained at this stage. The data for both *Meloidogyne* spp. differed from that by Fourie *et al*. (2009) who reported leveling off, and not a decline, of regression lines for both *Meloidogyne* spp. Even more interesting is that Fourie *et al*. (2009) used higher Pi levels (maximum 40 000 eggs and J2) compared to the highest of 10 000 used in this study. The most logical explanation for the decline in both *Meloidogyne* spp. populations in this study is that feeding was most probably restricted (due to damaged roots) for individuals of the two species at Pi 7 500 and 10 000, but to a lesser extent for *M*. *incognita* under the set of conditions under which these experiments were conducted. This phenomenon is known to occur especially when high population densities of *Meloidogyne* exist (Greco & Di Vito, 2009) or when the population experiences stress induced by either abiotic and/or biotic variables.

The reproduction potential of a mixed *Meloidogyne* spp. (70:30 ratio) for non-treated seed used in the second micro plot study was substantially higher than that of either the single-species of *M. incognita* and *M. javanica* populations used in the first micro plot study. The

substantially higher population densities at all Pi levels, confirms the susceptibility of cultivar DKC8010 to this mixed root-knot nematode population. No literature could, however, be found regarding the effect of Pi on Pf of mixed *Meloidogyne* spp. populations for maize. Results obtained for this study are, however, important since mixed populations of these two species are common in local maize production areas (Riekert, 1996; Riekert & Henshaw, 1998; Mc Donald *et al*., 2017).

Another objective of this study was to evaluate the effect of the abamectin seed treatment Avicta® 500FS (0.5 mg active substance / seed) on the population development of the mixed *Meloiodgyne* spp. population used in the micro plot study 2. The efficacy of this seed treatment on Pf levels of the latter *Meloidogyne* population was pronounced, although not always significantly different, compared to that of a non-treated control. For example, a 2.2 (micro plot study) to 2.5 (glasshouse study) times lower Pf was recorded for this treatment compared to that of the non-treated control. These results demonstrate what the value of using this seed treatment in reducing *Meloidogyne* spp. population densities in maize fields of local producers can be. Use of abamectin also has the benefits that it is a safer and cheaper product compared to the toxic Class I synthetically-derived nematicides registered in South Africa (Van Zyl, 2013). In South Africa, the cost of abamectin-treated seed is approximately R150.00 per hectare (Personal communication Mr Lukas Meyer, Syngenta South Africa, November 2017) compared to more that R500.00 per hectare for registered synthetically-derived nematicdes (Personal communication Prof. Driekie Fourie, North-West University, Potchefstroom, November 2017). It is, however, important to keep in mind that for both the microplot (semi-controlled conditions) and glasshouse (controlled conditions) studies of study the soil used was fumigated prior to planting. This could have influenced on the effect of observed for abamectin since natural interactions between microbes and the target nematode and abamectin was greatly ruled out. Field evaluations using different Pi levels should hence be done to conclude on the effect of abamectin on *Meloidogyne* spp. Although no literature could be traced relating the effect of abamectin on *Meloidogyne* in maize, Cabrera *et al*. (2009) reported that penetration rates of *Pratylenchus zeae* in maize roots (cultivar Liberal) was reduced between 50 and 80 % at a dosage rate of 0.6 mg a.i. of abamectin / seed, and more than 80 % at a dosage rate of 0.1 mg as. per seed. The latter authors illustrated the substantial contribution that of abamectin can make in reducing population densities of this species, which is also the predominant lesion nematode in South African maize production areas (De Waele and Jordaan, 1988; Mc Donald *et al*., 2017).

Since no yield data could be obtained for any of the three micro plot experiments conducted during this study, no correlations could be drawn between Pi levels and yield for the *Meloidogyne* spp. used. Fourie *et al*. (2009), however, demonstrated that yield losses for susceptible maize cultivars differed substantially, viz. 72 and 55 % for *M. incognita* and *M. javanica*, respectively, at Pi = 40 000. Such information is important for local maize cultivars grown under the reported changing climatic conditions (Bryan *et al.*, 2009). Trials should be done to generate information for producers about the effects of differential Pi levels of the predominant *Meloidogyne* spp. that prevail in South African maize production areas. The latter has been reported in Chapter 2, Paragraph 2.5.1.1, ranging from zero to a high 38 479 / 50 g roots.

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Chapter 4

4.1 Conclusions and recommendations

This study was initiated by requests from local maize (*Zea mays* L.) producers and the chemical industry, Syngenta SA (Pty), Ltd, since plant-parasitic nematodes result in substantial yield losses to this commodity in South Africa. These losses has been illustrated by nematode research done since the 1980s when Keetch (1989) listed 12 % yield losses for local maize crops, Riekert & Henshaw (1998) 60 % and Fourie *et al*. (2009) 72 %. Although the first author reported on the comprising effect of various nematode pests, the latter two focused on *Meloidogyne* Goeldi, 1892, which is regarded as the main problem-causing genus in local maize-based cropping systems (Riekert, 1996; Riekert & Henshaw, 1998; Mc Donald *et al*., 2017). These authors listed specifically *Meloidogyne incognita* (Kofoid & White, 1919) Chitwood, 1949 and *Meloidogyne javanica* (Treub, 1885) Chitwood, 1949 as the most abundant root-knot nematode species prevailing in maize fields, followed to a much lesser extent by *Meloidogyne arenaria* (Neal, 1889) Chitwood, 1949. *Pratylenchus* spp., and in particular *Pratylenchus zeae* Graham, 1951, are considered as the predominant migratory nematode pest genus in South African maize-based cropping systems (De Waele & Jordaan, 1988). Although various other plant-parasitic nematodes have been listed in association with local maize, their occurrence are usually restricted and their population densities low (Mc Donald *et al*., 2017).

The first part of this study comprised of a re-assessment of the plant-parasitic nematode pests occurring in South African maize fields but with special reference to *Meloidogyne*. This study represented the most extensive nematode survey being conducted to date in 78 fields of commercial producers. Identification of *M. incognita* as the predominant species (by a big margin of 99 % of the populations identified), followed by *M. javanica* (30 %) and to a much lesser extent *M. arenaria* (3 %) in these maize crop production areas, is in concurrence with existing literature (Riekert, 1996; Riekert & Henshaw, 1998; Bekker, 2016; Mc Donald *et al*., 2017). Novel information generated is that *M. enterolobii* was also identified in 1 % of local maize fields, which is, a first report for South Africa. The use of two molecular, deoxyribonucleic acid (DNA) techniques enabled species identification with the derived amplified region – polymerase chain reaction (SCAR-PCR) being the most accurate in identifying both single and mixed species populations from 71 of the 78 populations of which usable DNA could be obtained. By contrast, the NADH5 (using mitochondrial DNA) placed

the 68 local populations of which usable DNA could be obtained in the same clade as numerous other thermophilic *Meloidogyne* spp., which sequences were selected from NCBI Genbank. Hence no distinction between the selected species.. Concluding on the identification initiative is that that the SCAR-PCR should be the preferred molecular technique for identification of *Meloidogyne* spp. when such extensive surveys are done, due to it using species-specific markers that are most accurate. Molecular species identification is preferred to be complemented by morphological identification (Hunt & Handoo, 2009), which is agreed with. However, the nature of this survey did not allow the specialized and time-consuming preparation of 21 perineal patterns and corresponding oesophageal areas of females from each of the 78 localities sampled for morphometrical and morphological identification purposes for this MSc study. During the follow-up survey planned to be done on these same fields during 2018, morphological and morphometrical identification of selected populations, e.g. that of *M. enterolobii* specifically, will however be done to verify results.

Following *Meloidogyne*, *Pratylenchus* was identified as the second predominant nematode pest genus that infected maize. This is in contrast with results from the 1970's and 1980's when *Pratylenchus* was listed as the most abundant (De Waele & Jordaan, 1988). An explanation for this anomaly is that improvement of the adapted sodium hypochlorite method (NaOCl) by Riekert (1995) showed that optimal extraction of *Meloidogyne* spp. eggs and second-stage juveniles (J2) was isolated from maize roots with the latter species being proven to be predominant. Species identification was, however, not done for *Pratylenchus* during this study due to the extensive nature of the survey, which focused on *Meloidogyne*. Nonetheless, identification of *Pratylenchus* spp. is important and will be done during 2018 when a follow-up survey will be conducted at the same 78 localities.

The second part of this study focused on elucidating the nature of relationships between initial and final population densities (Pi and Pf, respectively) of the predominant *Meloidogyne* spp. determined, , in roots of the root-knot nematode susceptible cultivar DKC8010, by means of both micro plot and glasshouse studies. Substantial variation existed among the Pf levels of *M. incognita* and *M. javanica* in roots of this cultivar for the different Pi levels used. The Pf of *M. incognita* at the highest Pi level (10 000 eggs and J2 / root system) was substantially higher than that of *M. javanica*. This may, to a certain extent, explain why *M. incognita* is the predominant root-knot nematode species in local maize fields (Riekert, 1996; Riekert & Henshaw, 1998; Mc Donald *et al*., 2017), which was confirmed by the extensive

survey done (referred to above). Also interesting is the levelling off of Pf levels for *M. incognita* from only Pi 7 500, which was substantially higher than Pi 1 000 reported by Fourie *et al*. (2009) for susceptible cultivar AFG4410. By contrast, *M. javanica* Pf levels for the latter two studies were in a similar range. The sharp decline in the reproduction potential of *M. javanica* compared to that of *M. incognita* at Pi levels 7 500 and 10 000 eggs and J2 / root system, emanating from this study cannot be properly explained. This data differed from that by Fourie *et al*. (2009), who also used a higher Pf range and reported levelling off, and not a decline, of regression lines for both *Meloidogyne* spp. It is known that feeding of *Meloidogyne* is restricted when high population densities occur (Sasser *et al.,* 1975) due to a lack of feeding sites, which was most probably the case in this study under the set of conditions these trials were conducted. Differences experienced for Pi between these studies might, however, be attributed to the different *M. incognita* populations as well as the different maize cultivars used as had been reported in the literature (Anwar *et al*., 2000; Ngobeni *et al*., 2010). Except for these factors, differences in abiotic conditions may also impact on results of different studies. Ultimately, no concrete conclusion can be made in this regard since additional research is need to elucidate this scenario.

Another interesting finding was that the reproduction potential of a mixed *M. incognita* and *M. javanica* (70:30 ratio) population for non-treated seed used in a second micro plot study was substantially higher than that of either of the single-species populations (first micro plot study). Ultimately, the substantially higher Pfs at all Pi levels confirms the susceptibility of cultivar DKC8010 to the single and mixed *Meloidogyne* spp. population used in this study. Although no literature could be found about the effect of Pi on Pf of mixed *Meloidogyne* spp. populations for maize, results of this study are important since mixed populations of these two species commonly occur in local maize production areas (Riekert, 1996; Riekert *&* Henshaw, 1998; Mc Donald *et al*., 2017). A definite draw-back of this study was that no yield data could be obtained (due to unforeseen circumstance beyond the control of the student) for any of the three micro plot experiments conducted during this study. Therefore, no correlations could be drawn between Pi levels and yield for the *Meloidogyne* spp. used which is crucial information for producers to enable optimal management of these nematode pests.

Pronounced decreases in Pfs of the mixed *Meloidogyne* spp. population for the abamectin seed treatment Avicta® 500FS (0.5 mg active substance / seed), 2.2 (micro plot study) and 2.5 (glasshouse study) times lower compared to that of a non-treated control were also not

unexpected. These results confirmed the potential value of using this seed treatment in reducing *Meloidogyne* spp. population densities in local maize fields. This product is safer and cheaper compared to the toxic Class I synthetically-derived nematicides registered in South Africa (Van Zyl, 2013) and represents a cost-effective alternative. However, it is important to note that the soil used for this study was fumigated prior to planting, which could have influenced on the effect observed for abamectin. Field evaluations should hence be done to verify these results.

Some results from this study ultimately represent novel and useful information to assist local producers and the related crop, chemical and seed industries to be pro-active in managing root-knot nematode pests in particular optimally.

4.2 References:

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Annexure

Table 2.10 Prominence values (PVs) of plant-parasitic nematode species per 200g soil samples for 78 fields sampled during the 2013/14 growing season. *Meloidogyne* spp.

Sample ID	Prominence values (PV)	Frequency of occurence	Mean population density (MPD)	Sample ID	Prominence values (PV)	Frequency of occurence	Mean population density (MPD)
1	23	75	27	40	7	25	13
2	0	0	0	41	2	25	3
3	14	38	23	42	9	63	12
4	0	1	1	43	215	50	304
5	0	0	0	44	0	13	1
6	1	13	4	45	0	0	0
7	28	63	35	46	0	0	0
8	5	38	8	47	2	25	3
9	6	50	8	48	85	38	139
10	1	38	2	49	0	0	0
11	69	88	74	50	6	38	10
12	0	0	0	51	0	0	0
13	0	0	0	52	0	0	0
14	0	0	0	53	1	13	2
15	4	38	6	54	2	25	3
16	2	25	3	55	285	88	305
17	5	25	9	56	390	100	390
18	0	13	1	57	187	100	187
19	62	88	66	58	0	13	1
20	32	63	40	59	1	25	2
21	1	25	18	60	0	13	1
22	0	0	0	61	0	0	0
23	103	63	145	62	1	13	3
24	2	25	3	63	1	25	2
25	51	38	83	64	0	0	0
26	13	63	17	65	0	0	0
27	15	25	30	66	0	13	1
28	5	25	10	67	0	0	0
29	0	0	0	68	1	25	2
30	3	38	5	69	45	25	90
31	2	13	5	70	0	0	0
32	0	0	0	71	205	75	237
33	1	1	2	72	3	50	4
34	6	38	10	73	3	25	5
35	0	0	0	74	50	38	82
36	0	0	0	75	11	13	30
37	3294	100	3294	76	1	13	3
38	4	25	8	77	116	88	124
39	167	88	179	78	4	25	8

Table 2.11 Prominence values (PVs) of plant-parasitic nematode species per 200g soil samples for 78 fields sampled during the 2013/14 growing season. *Pratylenchus* spp.

Sample ID	Prominence values (PV)	Frequency of occurence	Mean population density (MPD)	Sample ID	Prominence values (PV)	Frequency of occurence	Mean population density (MPD)
1	34	100	34	**40**	364	100	364
2	15	75	17	**41**	63	100	63
3	38	88	40	**42**	14	88	15
4	84	88	90	**43**	128	100	128
5	8	75	9	**44**	63	88	67
6	62	88	66	**45**	232	100	232
7	54	88	58	**46**	9	75	10
8	96	100	96	**47**	49	100	49
9	42	100	42	**48**	88	100	88
10	22	88	23	**49**	26	63	33
11	78	100	78	**50**	77	100	77
12	34	88	36	**51**	214	100	214
13	25	50	35	**52**	26	75	30
14	53	100	53	**53**	253	100	253
15	55	100	55	**54**	94	88	100
16	67	100	67	**55**	91	100	91
17	52	88	55	**56**	80	88	85
18	9	88	10	**57**	16	88	17
19	62	88	66	**58**	5	63	6
20	205	100	205	**59**	397	100	397
21	15	88	16	**60**	245	100	245
22	20	100	20	**61**	52	100	52
23	59	100	59	**62**	116	100	116
24	68	100	68	**63**	465	100	465
25	90	100	90	**64**	18	63	23
26	39	88	42	**65**	12	63	15
27	31	75	36	**66**	22	63	28
28	14	88	15	**67**	396	100	396
29	57	63	72	**68**	28	100	28
30	25	88	27	**69**	304	100	304
31	210	88	224	**70**	156	100	156
32	109	88	116	**71**	220	100	220
33	157	88	167	**72**	9	63	12
34	38	75	44	**73**	5	50	7
35	275	100	275	**74**	99	100	99
36	64	100	64	**75**	463	100	463
37	108	88	115	**76**	59	88	63
38	328	100	328	**77**	76	100	76
39	101	88	108	**78**	78	100	78

Table 2.12 Prominence values (PVs) of plant-parasitic nematode species per 200g soil samples for 78 fields sampled during the 2013/14 growing season. *Helicotylenchus* spp.

Sample ID	Prominence values (PV)	Frequency of occurence	Mean population density (MPD)	Sample ID	Prominence values (PV)	Frequency of occurence	Mean population density (MPD)
1	1	25	2	**40**	89	88	95
2	7	63	9	**41**	13	88	14
3	108	100	108	**42**	7	50	10
4	53	100	53	**43**	25	75	29
5	18	75	21	**44**	39	50	55
6	2	38	3	**45**	2	25	3
7	1	13	2	**46**	0	0	0
8	79	88	84	**47**	9	75	10
9	2	25	4	**48**	64	100	64
10	48	75	55	**49**	132	100	132
11	55	75	64	**50**	33	88	35
12	22	88	23	**51**	49	75	57
13	153	100	153	**52**	23	50	32
14	46	75	53	**53**	92	88	98
15	89	100	89	**54**	23	75	27
16	82	100	82	**55**	47	63	59
17	1	25	2	**56**	30	88	32
18	14	88	15	**57**	2	50	3
19	92	100	92	**58**	17	88	18
20	70	100	70	**59**	59	88	63
21	10	63	13	**60**	551	100	551
22	9	75	10	**61**	493	100	493
23	37	75	43	**62**	3	38	18
24	91	100	91	**63**	367	88	392
25	6	25	11	**64**	256	100	256
26	3	38	4	**65**	0	0	0
27	90	100	90	**66**	5	13	13
28	14	75	16	**67**	103	75	119
29	4	38	6	**68**	20	88	21
30	1	25	1	**69**	51	63	64
31	161	100	161	**70**	75	88	80
32	23	50	32	**71**	47	63	60
33	211	88	225	**72**	36	75	41
34	165	100	165	**73**	0	0	0
35	196	100	196	**74**	9	63	11
36	51	100	51	**75**	195	100	195
37	5	13	13	**76**	9	38	15
38	555	88	593	**77**	55	75	69
39	0	0	0	**78**	71	75	82

Table 2.13 Prominence values (PVs) of plant-parasitic nematode species per 200g soil samples for 78 fields sampled during the 2013/14 growing season. *Criconema* spp.

Sample ID	Prominence values (PV)	Frequency of occurence	Mean population density (MPD)	Sample ID	Prominence values (PV)	Frequency of occurence	Mean population density (MPD)
1	1	25	1	**40**	1	13	3
2	0	0	0	**41**	124	88	132
3	1	38	2	**42**	0	0	0
4	1	13	4	**43**	23	75	27
5	0	0	0	**44**	30	50	43
6	1	13	3	**45**	140	75	162
7	0	0	0	**46**	73	88	78
8	0	13	1	**47**	0	0	0
9	4	38	6	**48**	0	0	0
10	0	13	1	**49**	0	0	0
11	3	25	5	**50**	0	0	0
12	1	13	2	**51**	0	0	0
13	0	0	0	**52**	1	25	1
14	0	0	0	**53**	3	38	5
15	1	25	2	**54**	6	50	5
16	0	13	1	**55**	0	0	0
17	3	25	5	**56**	0	0	0
18	1	25	2	**57**	0	0	0
19	0	0	0	**58**	1	13	2
20	0	0	0	**59**	3	25	5
21	1	13	3	**60**	9	63	11
22	0	0	0	**61**	0	0	0
23	0	0	0	**62**	93	88	99
24	0	0	0	**63**	1	13	2
25	0	13	1	**64**	0	0	0
26	22	38	36	**65**	72	88	77
27	0	0	0	**66**	6	50	5
28	2	25	3	**67**	3	25	5
29	0	0	0	**68**	2	25	3
30	7	63	9	**69**	7	38	12
31	1	25	2	**70**	0	0	0
32	1	13	2	**71**	8	38	13
33	9	25	18	**72**	28	88	30
34	72	75	83	**73**	174	100	174
35	0	0	0	**74**	25	75	29
36	0	0	0	**75**	0	0	0
37	1	13	2	**76**	0	13	1
38	0	0	0	**77**	0	0	0
39	21	38	35	**78**	45	63	57

Table 2.14 Prominence values (PVs) of plant-parasitic nematode species per 200g soil samples for 78 fields sampled during the 2013/14 growing season. *Rotylenchulus* spp.

Sample ID	Prominence values (PV)	Frequency of occurence	Mean population density (MPD)	Sample ID	Prominence values (PV)	Frequency of occurence	Mean population density (MPD)
1	0	0	0	**40**	0	0	0
2	0	0	0	**41**	2	25	3
3	2	38	3	**42**	0	0	0
4	35	38	57	**43**	21	63	27
5	0	0	0	**44**	1	25	2
6	0	0	0	**45**	0	13	1
7	0	0	0	**46**	0	0	0
8	0	0	0	**47**	1	13	2
9	0	0	0	**48**	15	50	21
10	22	75	25	**49**	278	88	297
11	6	25	9	**50**	0	0	0
12	4	38	7	**51**	7	50	10
13	23	50	32	**52**	3	25	6
14	0	13	1	**53**	10	25	19
15	1	25	1	**54**	3	25	5
16	38	88	41	**55**	0	0	0
17	0	0	0	**56**	0	0	0
18	0	0	0	**57**	2	13	6
19	0	0	0	**58**	0	0	0
20	0	0	0	**59**	28	38	46
21	0	0	0	**60**	306	100	306
22	0	0	0	**61**	261	100	261
23	0	0	0	**62**	4	25	7
24	0	0	0	**63**	0	0	0
25	0	0	0	**64**	106	100	106
26	0	0	0	**65**	0	0	0
27	3	13	7	**66**	0	13	1
28	0	0	0	**67**	0	0	0
29	0	0	0	**68**	1	13	2
30	0	0	0	**69**	2	25	3
31	21	63	26	**70**	232	25	464
32	0	0	0	**71**	9	38	14
33	6	50	9	**72**	0	0	0
34	15	38	24	**73**	0	0	0
35	32	50	45	**74**	3	13	8
36	100	88	107	**75**	0	0	0
37	14	38	22	**76**	432	100	432
38	1	13	2	**77**	0	0	0
39	475	88	508	**78**	29	63	36

Table 2.15 Prominence values (PVs) of plant-parasitic nematodes per 200g soil samples for 78 fields sampled during the 2013/14 growing season. Family Trichodoridae (either *Nanidorus* and/or *Paratrichodorus*)

Sample ID	Prominence values (PV)	Frequency of occurence	Mean population density (MPD)	Sample ID	Prominence values (PV)	Frequency of occurence	Mean population density (MPD)
1	0	0	0	**40**	0	13	1
2	0	0	0	**41**	0	0	0
3	0	0	0	**42**	0	0	0
4	0	0	0	**43**	1	13	3
5	0	0	0	**44**	0	0	0
6	0	0	0	**45**	0	0	0
7	0	0	0	**46**	0	0	0
8	0	0	0	**47**	0	0	0
9	0	0	0	**48**	0	0	0
10	1	13	2	**49**	0	0	0
11	0	0	0	**50**	0	0	0
12	0	0	0	**51**	0	0	0
13	0	0	0	**52**	0	0	0
14	0	0	0	**53**	0	0	0
15	0	0	0	**54**	0	0	0
16	0	0	0	**55**	0	0	0
17	0	13	1	**56**	0	13	1
18	0	0	0	**57**	0	0	0
19	0	0	0	**58**	0	0	0
20	0	0	0	**59**	0	0	0
21	0	0	0	**60**	1	13	3
22	0	0	0	**61**	0	0	0
23	0	0	0	**62**	0	0	0
24	0	0	0	**63**	1	13	2
25	0	0	0	**64**	0	13	1
26	0	0	0	**65**	0	0	0
27	0	0	0	**66**	0	0	0
28	0	0	0	**67**	3	13	9
29	0	0	0	**68**	0	0	0
30	0	0	0	**69**	0	0	0
31	0	0	0	**70**	0	0	0
32	0	0	0	**71**	1	25	2
33	0	0	0	**72**	0	0	0
34	0	0	0	**73**	0	0	0
35	0	0	0	**74**	0	0	0
36	0	0	0	**75**	4	50	6
37	0	0	0	**76**	0	0	0
38	0	0	0	**77**	0	0	0
39	0	0	0	**78**	0	0	0

Table 2.16 Prominence values (PVs) of plant-parasitic nematode species per 200g soil samples for 78 fields sampled during the 2013/14 growing season. *Tylenchorhynchus* spp.

Sample ID	Prominence values (PV)	Frequency of occurence	Mean population density (MPD)	Sample ID	Prominence values (PV)	Frequency of occurence	Mean population density (MPD)
1	1	13	4	**40**	0	0	0
2	0	0	0	**41**	3	13	7
3	0	0	0	**42**	4	50	6
4	0	0	0	**43**	0	13	1
5	0	0	0	**44**	0	13	1
6	0	13	1	**45**	0	0	0
7	3	38	5	**46**	0	0	0
8	1	13	4	**47**	1	13	2
9	0	0	0	**48**	0	0	0
10	0	0	0	**49**	0	0	0
11	0	0	0	**50**	32	75	37
12	0	13	1	**51**	0	0	0
13	0	0	0	**52**	0	0	0
14	0	13	1	**53**	3	25	6
15	0	0	0	**54**	1	13	2
16	0	0	0	**55**	5	50	7
17	0	13	1	**56**	18	88	19
18	0	13	1	**57**	0	13	1
19	2	38	3	**58**	0	0	0
20	8	25	15	**59**	0	0	0
21	0	0	0	**60**	0	0	0
22	0	0	0	**61**	0	0	0
23	4	38	7	**62**	0	0	0
24	0	0	0	**63**	0	0	0
25	0	0	0	**64**	0	0	0
26	6	63	8	**65**	0	13	1
27	0	0	0	**66**	0	0	0
28	23	88	25	**67**	0	0	0
29	0	0	0	**68**	0	0	0
30	0	0	0	**69**	0	0	0
31	0	0	0	**70**	0	0	0
32	4	38	6	**71**	1	25	2
33	0	0	0	**72**	0	0	0
34	0	0	0	**73**	0	0	0
35	0	0	0	**74**	0	0	0
36	0	0	0	**75**	0	0	0
37	0	0	0	**76**	0	0	0
38	0	0	0	**77**	16	63	20
39	0	0	0	**78**	0	0	0

Table 2.17 Prominence values (PVs) of plant-parasitic nematode species per 200g soil samples for 78 fields sampled during the 2013/14 growing season. *Xiphinema* spp.

Sample ID	Prominence values (PV)	Frequency of occurence	Mean population density (MPD)	Sample ID	Prominence values (PV)	Frequency of occurence	Mean population density (MPD)
1	0	0	0	40	0	0	0
2	0	0	0	41	0	0	0
3	0	0	0	42	0	0	0
4	0	0	0	43	0	13	1
5	0	0	0	44	0	0	0
6	0	0	0	45	0	0	0
7	0	0	0	46	0	0	0
8	0	0	0	47	0	0	0
9	0	0	0	48	0	0	0
10	0	0	0	49	0	0	0
11	0	0	0	50	0	0	0
12	0	0	0	51	0	0	0
13	0	0	0	52	0	0	0
14	0	0	0	53	12	75	14
15	0	0	0	54	1	13	2
16	0	0	0	55	0	0	0
17	0	0	0	56	0	0	0
18	0	0	0	57	0	0	0
19	0	0	0	58	0	0	0
20	0	0	0	59	0	0	0
21	0	0	0	60	0	0	0
22	0	0	0	61	0	0	0
23	0	0	0	62	0	0	0
24	0	0	0	63	0	0	0
25	0	0	0	64	0	0	0
26	0	0	0	65	0	0	0
27	0	0	0	66	0	0	0
28	0	0	0	67	0	0	0
29	0	0	0	68	0	0	0
30	0	0	0	69	3	38	4
31	0	0	0	70	21	88	22
32	0	0	0	71	0	0	0
33	2	25	3	72	0	0	0
34	0	0	0	73	0	0	0
35	0	0	0	74	0	0	0
36	0	0	0	75	2	25	3
37	0	0	0	76	3	38	4
38	0	0	0	77	0	0	0
39	0	0	0	78	0	0	0

Table 2.18 Root mass and areal plant mass data of a root-knot nematode susceptible maize cultivar of which a batch of seeds were treated with Avicta® 500FS and another not treated (control) to determine their effect on different initial inoculation levels of a mixed *Meloidogyne incognita* and *Meloidogyne javanica* (70:30 ratio) population.

Pi level	Root mass: Non-treated seeds	Root mass: Avicta treated seeds	Aerial mass: Non-treated seeds	Aerial mass: Avicta treated seeds
0	62.1 ±11.8aA	60.6 ±7.6aA	208.6 ±29.0aA	240.3 ±22.9aA
50	60.2 ±7.7aA	51.7 ±8.6aA	210.7 ±27.1aA	218.1 ±32.2aA
100	54.1 ±8.0aA	48.0 ±7.3aA	203.8 ±25.7aA	194.2 ±27.2aA
500	56.9 ±7.9aA	57.6 ±9.6aA	204.5 ±27.2aA	215.6 ±24.2aA
1 000	63.5 ±10.9aA	50.7 ±6.2aA	209.6 ±33.8aA	199.1 ±22.9aA
5 000	55.2 ±6.2aA	56.9 ±10.1aA	206.0 ±27.7aA	211.7 ±24.7aA
P value	0.835	0.590	1.000	0.149
F ratio	0.414	0.756	0.029	1.800
Interaction data: Non-treated vs treated seeds				
P value = 0.821			P value = 0.849	
F ratio = 0.437			F ratio = 0.396	

*Log (x+1) transformed data; **Real means; ***Standard Error; means in the same column followed by the same lower case letter do not differ significantly ($P \leq 0.05$) according to Tukey's HSD Test, while means in the same row followed by the same uppercase letter do not differ significantly ($P \leq 0.05$) according to Tukey's HSD Test.

Table 2.20 Cultivar names of maize grown at the localities where a nematode survey was undertook in South Africn maize production areas during the 2013/14 growing season.

Sample ID	Name of farmer	Cultivar planted	Sample ID	Name of farmer	Cultivar planted
1	Calby Farming	Info not attained	40	J du Rant	73 74 Monsanto
2	C Forbes	Info not attained	41	AC van Wyk	Info not attained
3	G Mulder	35 05	42	H Conradi	PHI 31 M 07
4	D Wensel	73 74	43	J Suurd	Info not attained
5	Jappie VTV Bdy	Info not attained	44	J du Plessis	Info not attained
6	M Labouschagne	Info not attained	45	P Ferreira	Info not attained
7	William Easby	78 17 Mon	46	J Minnaar	DKC 78 45 BR Gen
8	M Allan	30Y85 Pioneer	47	L Deale	78 17 DKC
9	M Erasmus	Info not attained	48	P Becker	Monsanto 78 45
10	B Haasbroek	Info not attained	49	D Schoeman	PAN 6Q408 BT
11	B Rheeder	Rondevlei PAN 5685	50	K du Preez	Info not attained
12	D de Water	PHI 33H56	51	C Cronje	Info not attained
13	K Marais	Decalp 7374	52	W Venter	73 74 Monsanto
14	J Breytenbach	DKC 80 30 R	53	L van Wyk	Info not attained
15	H Prinsloo	PAN 6Q245	54	J Basson	DKC 73 74 BR
16	J Randall	Bio Gene 42 96	55	F van der Merwe	Info not attained
17	S Becker	Info not attained	56	K du Preez	Info not attained
18	H Gouws	Info not attained	57	D du Toit	Info not attained
19	H Prinsloo	32 BIO PHI	58	P du Plessis	78 45
20	P du Plessis	32B10	59	C Cronje	Dekalb 73 70 B GEN.
21	Cobus Wentzel	76 07 RWVNZ	60	D Cronje	33H65 PHI
22	N Botha	62 80 Monsanto	61	H de Beer	78 35 Monsanto
23	K Human	PAN 6126	62	P Ferreira	DKC 78 45 BR
24	M Allan	6Q345 Pannar	63	D Oosthuizen	DKC 73 74 BR
25	D Berg	Info not attained	64	C Opperman	Info not attained
26	A Fourie	78 15	65	J Fourie	Dekalb 78 79 BR
27	K le Roux	42 96 Biogene	66	D Viljoen	78 17 Monsanto
28	P Roux	78 45 BR Gen	67	H Muller	73 74 DKC BR
29	G Mulder	77 61B Monsanto	68	K Muller	Info not attained
30	C Muller	73 74 Monsanto BR	69	B van Wyk	DK 80 40 BR
31	M van Rooyen	73 74 Monsanto	70	P Carrol	80 30 Monsanto
32	J Grobler	Info not attained	71	F van Rooyen	PAN 445 B
33	J Basson	DKC 73 76 R	72	JP Meintjies	Dekalb 77 77 BR
34	J van Dyk	Info not attained	73	J Potgieter	Dekalb 7845
35	S Bosman	Info not attained	74	C Muller	73-74 Monsanto BR
36	H Wapenaar	73 70 Monsanto DKC	75	W Venter	78-79 Monsanto
37	G Cronje	77 85 Monsanto	76	D Myburg	Info not attained
38	N Leslie	DKC 73 74 BR GEN	77	A du Preez	PAN 6528/5628 B
39	G Cronje	77 85 Monsanto	78	H Prinsloo	Info not attained