

# **Evaluation of integrated pest management practices on nematode assemblages in a potato field in South Africa**

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Dissertation accepted in fulfillment of the requirements for the degree *Master of Science in Environmental Sciences* at the North-West University

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Graduation May 2022

28385195

## ACKNOWLEDGEMENTS

I dedicate this study to the Lord Almighty. He has blessed me beyond all comprehension and I am humbled by Him and thankful for His hand covering me. I also dedicate this study to my husband, Pieter and our beautiful daughters, Jana and Liani.

To my husband, thank you for all your support (providing the trial site, site preparations, plantings and harvesting and so much more) and for helping me make it through to the end.

I want to specially thank my lovely supervisors. Your advice, patience and understanding during this trial, both academically and personally have been invaluable.

I would also like to thank the following people for their support, love and kindness:

- My parents, Steven and Latricia, for your never-ending love and support and for pushing me to pursue my dreams.
- My sister, Stephnè, for your love, motivation and optimism.
- My father-in-law, Pieter, for his continuous interest in my endeavours and diligent record-keeping. Our conversations help keep my 'research-mind' sharp.
- Both my mothers-in-law, Lynn and Carin, for their optimism, love, support and kind words when my positivity was low.
- Dr. Milad Rashidifard, Dr. Gerhard du Preez and Raymond Collett at NWU, for your support in the lab. Without your assistance, I would not have been able to finish this study.
- My colleagues at Bayer, for your support, advice, kind words and positivity.
- The late Prof. Terry Aveling. Your belief, love of life and support have made a very deep impact on my life. I have been able to reach this point in my life due to the passion for agriculture and nematodes you have sparked.

## ABSTRACT

Mounting pressure to reduce the use of chemical pesticides globally necessitated this study to investigate the potential of a neem-amended organic manure product (Kalahari 3:1) to aid potato farmers in reducing plant-parasitic nematode (PPN) population densities and the subsequent damage they cause. The efficacy of an organic neem-amended manure product was compared to that of registered chemical nematicides in combination with grass cover cropping using White Buffalo grass (*Urochloa mosambicensis* (Hack.) Dandy.), representing a natural fallow period, in a field near Tzaneen, Limpopo province, during 2018-2021. The field was planted with the cultivar Sifra. Plant-parasitic and beneficial soil nematodes were extracted from roots and soil at four sampling intervals (pre-plant, 10 weeks after emergence, harvest and after fallow). Root-knot nematodes (RKN) were identified to species level, using morphological and SCAR-PCR techniques, indicating that *Meloidogyne javanica* and *M. enterolobii* were the predominant RKN species present in the trial site. The presence of *M. enterolobii* on potato in Tzaneen, Limpopo, is therefore, a new addition to the South African Plant Parasitic Nematode Survey (SAPPNS) database. Contrary to literature and expectations, Kalahari 3:1 (stand-alone or combined with chemical nematicides) was not able to reduce population densities of predominant PPN genera (*viz.* RKN, lesion, spiral and ring nematodes) in the soil or roots. The combination of Nemacur® 400 EC with Velum® Prime 400 SC provided the best control against PPN in roots and soil in this study. The use of Kalahari 3:1, a stand-alone treatment, did have a more positive effect on beneficial soil nematodes during the potato crop cycle than the standard nematicides. Increased beneficial nematode community composition and abundance, especially bacterivore abundance, were observed. Soil nematode communities were not related to soil physico-chemical properties in this trial. After a 27-month grass fallow period, the PPN abundance declined and bacterivore nematode abundance increased from pre-plant level. However, the fallow period and grass cover crop did not improve the overall soil health or soil nematode community and more interventions would be required to improve the health of this field.

Key words: neem-amended manure; alternative plant-parasitic nematode control; potato; soil health; bioindicators.

## **1 INTRODUCTION**

The guidelines set out in this guide is based on the APA Publication Manual, seventh edition, published in October 2019. It is recommended to use this guide in conjunction with the seventh edition of the APA Publication Manual for full details on APA publishing.

The APA style is an author-date citation style. This implies that a short reference (author and date) is made in the text, with a corresponding entry in the alphabetical reference list, which provides more detail of the source being used. Each text reference must have a corresponding entry in the reference list, and each entry in the reference list must have a text reference.

## **REFERENCING**

The APA referencing style was used, which places citations in alphabetical order of the author's surnames, from the 2020 revised NWU Referencing Guide, which is available at <https://library.nwu.ac.za/referella>.

## **ETHICS DECLARATION**

No need for ethical clearance: Research does not involve human participants, animals, with as little as possible environmental impact or risk to the researcher.

Ethics number: NWU-01190-19-A9

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## **Chapter 1: Introduction and literature review**

### **1.1 Introduction**

With mounting pressure, from regulatory authorities worldwide, to reduce the use of certain pesticides, the management of plant-parasitic nematodes (PPN) has become more difficult. To circumvent this, producers and scientists have been investigating different alternative nematode management strategies. Potato (*Solanum tuberosum* L.) is one of the crops that are crucial in terms of food security, taking into consideration the rapid human population growth and increased hunger rates (Stokstad, 2019). This crop is ranked the third largest staple food crop in South Africa in terms of production, with a gross value of nearly ZAR 7.76 billion (FAOSTAT, 2020a). Seed and table potato crops are exposed to severe damage caused by various diseases and pests such as PPN of which root-knot nematodes (RKN; *Meloidogyne* Goeldi, 1982), followed by lesion nematodes (LN; *Pratylenchus* Filipjev, 1936) and the golden potato cyst nematode (GCN; *Globodera rostochiensis* (Wollenweber, 1923; Skarbilovich, 1959) are economically the most important nematodes that infect and damage the crop in South Africa (Jones *et al.*, 2017; Kleynhans *et al.*, 1996). Similarly, these nematode genera and/or species are generally listed as the most damaging in other countries, although the order in which they are listed in this regard may differ (Niere & Karuri, 2018).

Extensive damage caused by PPN to potato is experienced, despite the widespread use of synthetic nematicides (Jones *et al.*, 2017; Niere & Karuri, 2018). Proper management of PPN is crucial to ensure the continued productivity of the potato industry with good quality produce. Research studies to address the PPN problem in optimising sustainable potato production are essential for local producers, related industries and the economy. Since RKN and LN have very wide host ranges (Kleynhans *et al.*, 1996; Moens & Perry, 2009), these nematode genera are also detrimental to the production of other crops that are planted in rotation with potato.

### **Hypothesis, aim and objectives of the study**

The increased challenge faced by local potato producers to reduce PPN numbers in their fields prompted this study and was further necessitated by the removal of chemical nematicides from the markets. The way forward is more environmentally friendly strategies and therefore, this study focussed on an organic fertiliser granule incorporated with neem seed oil. The envisaged results will provide producers and the potato industry with a more sustainable program to reduce root-knot nematode population densities and other PPN as well as improve soil health.

### **Hypothesis**

Application of an easy-to-use, granulated cow manure amended with neem oil (Kalahari 3:1) is expected to offer nematicidal activity, comparable to that of registered nematicides, against PPN

infecting potato under typical South African field conditions, while increasing tuber quality. The use of Kalahari 3:1 succeeded by a natural grass fallow is also foreseen to improve soil health, resulting in higher abundance and more diverse beneficial soil nematode communities compared to nematicide-treated areas.

## **Aim**

To assist in contributing towards sustainable potato production in South Africa by investigating the effect of an organic nematicidal product compared to existing, registered, chemical products on nematode assemblages in a potato field (situated in the Tzaneen area, Limpopo province, South Africa) in combination with grass cover cropping.

## **Objectives**

The specific objectives of this study were to investigate whether:

- i) an environmentally friendly nematicidal product, Kalahari 3:1 (neem-amended cow manure granule), is effective in reducing the population density of predominantly occurring PPN species, both when applied alone and in various combinations with two registered, chemical nematicide products;
- ii) the quality and yield of the potato crop will be improved by the alternative product's application and
- iii) this product as well as a grass cover cropping impact positively on beneficial nematode assemblages that were used as bioindicators of soil health.

## **1.2 Potato (*Solanum tuberosum* L.)**

### **1.2.1 Origin**

Potato originated from Mexico and spread across the south-western United States and South America (Argentina and Chile) where it was domesticated in the highland of southern Peru (Bradshaw & Ramsay, 2009; Spooner *et al.*, 2014). It is not clear how this crop was introduced to South Africa, but it has been suggested that potato tubers were brought from Holland to the Cape in the early 1600s to be planted as food for seafarers (DAFF, 2013; FAO, 2008).

### **1.2.2 Classification**

The potato belongs to the family Solanaceae. The full classification is as follows (CABI, 2019; Patil *et al.*, 2016):

Kingdom: Plantae  
Phylum: Anthophyta  
Class: Magnoliopsida  
Order: Solanales

Family: Solanaceae  
Genus: *Solanum*  
Species: *Solanum tuberosum* L.

### 1.2.3 Anatomy of the potato

Potato, an annual, herbaceous-type plant of which only the tubers are edible, can grow under a broad spectrum of geographical and climatic conditions (Ramadan & Oraby, 2016). It shares the genus *Solanum* with at least 1 000 other species, including tomato (*Solanum lycopersicum* L.) and eggplant (*Solanum melongena* L.) (Datiles & Acevedo-Rodriguez, 2014; Hawkes, 1990). The tuber contains carbohydrates, vitamins, protein, small molecules and secondary metabolites and is valued for its high starch content (Ezekiel *et al.*, 2013). Tubers are consumed fresh (medium-small sizes) or sold for processing (large – large-medium sizes) into instant mashed potato, frozen fries, mixed frozen vegetables and other forms. Potato varieties are categorised into four colour groups, namely red, white, yellow or purple/blue (Jovovic *et al.*, 2013; Potatoes USA, 2019). Standardised quality classifications for potato are namely Class 1 (highest quality), Class 2, Class 3 and Lowest class (rejects) and prices are linked to class as well as tuber size (DAFF, 2015).

### 1.2.4 Economical and social importance of the potato

Potato is the fourth most important crop in the world, exceeded only by maize (*Zea mays* L.) (1147 million tons), wheat (*Triticum aestivum* L.) (734 million tons) and rice (*Oryza sativa* L.) (782 million tons) (Stokstad, 2019), with an annual production of over 368 million tons in 2018 (FAOSTAT, 2020b). In addition, potato is the third most widely consumed plant product by humans after wheat and rice, making it a staple food in most countries (Aliche *et al.*, 2020; Stokstad, 2019). According to Potato South Africa (PSA) (PSA, 2019), the six largest potato producing countries in Africa in 2017 were Algeria, Egypt, South Africa, Morocco, Malawi and Rwanda.

### 1.2.5 Potato production in South Africa

Potato production in South Africa takes place in 16 regions (Figure 1.1). These regions differ in some key facets, such as climate, soil types, planting times and cultivars planted, with the Limpopo, Free State, KwaZulu-Natal provinces and the Sandveld area being the most important production areas (Haverkort *et al.*, 2013; Van der Waals *et al.*, 2016). According to PSA (2019), potato contributes 2.69% of the total gross production value of South African agriculture. In 2019, South Africa was estimated to be the third largest potato producer (2 666 994.65 tons produced in 2020) in Africa with the highest tonnage per hectare (37 t/ha) compared to other countries listed in the top six. This comprised 10% of the potatoes produced in Africa (PSA, 2021). During the last decade, about 50 000-55 000 ha, representing approximately 1% of available arable land, have been used for potato production (PSA, 2020) producing between 245 and 250 million bags

(10 kg each) depending on climatic conditions. Producers have been increasing potato yield without significantly increasing the size of the production area (Jooste, 2019; PSA, 2019). This was mostly accomplished by improving production practices including increased soil health, improved yield stability and planting disease resistant and/or tolerant cultivars although producers also utilise newly cleared land or use fields previously used for other crops to increase production (Kleinwechter *et al.*, 2016; Litaladio *et al.*, 2009; Mallory & Porter, 2007).

Fresh potato is available for consumption throughout the year (DAFF, 2013) since the crop is planted at different times of the year (Autumn-Summer) due to climatic differences in the 16 production areas (Figure 1.1). Rainfall in these areas ranges from approximately 224-1140 mm and producers utilise both dryland and irrigation practices, depending on the area. Some of the most popular cultivars planted in these areas include Mondial (53%), Sifra (26%), Lanorma (7%) and Valor (3%) (PSA, 2021).

Potato growers in South Africa struggle with a variety of problems of both a biotic and abiotic nature. Some of the abiotic factors includes erratic rainfall or drought, hail, frost, extreme temperatures and other climatic extremes (Aliche *et al.*, 2020; Steyn *et al.*, 2016; Van der Waals *et al.*, 2016). These types of factors may vary greatly in a single season, depending on which of the 16 potato growing areas in South Africa is in question. The untimely occurrence of too much rain at a single point during the growing season is perceived as the biggest abiotic issue that producers struggle with in certain areas (Haverkort *et al.*, 2013; Van der Waals *et al.*, 2016). On the other hand, the lack of sufficient, good quality irrigation water in other areas is also a major restriction, limiting production potential and yield (Franke *et al.*, 2011; Steyn *et al.*, 2016; Van der Waals *et al.*, 2016).

Biotic factors include a wide variety of pests and diseases that negatively affect potato production. Diseases caused by fungi, bacteria and viruses threaten the potential productivity and sustainability of local potato production (Van der Waals *et al.*, 2013 & 2016). Fungal diseases include early and late blight (*Alternaria solani* Sorauer, 1896 and *Phytophthora infestans* (Mont.) de Bary, 1876), brown spot (*Alternaria alternata* (Fr.) Keissl., 1912), black and silver scurf (*Rhizoctonia solani* Kühn, 1858), *Helminthosporium solani* (Durieu & Mont., 1849), Fusarium and Verticillium wilts (*Fusarium* Link, 1809, *Verticillium dahlia* Kleb, 1913), *V. albo-atrum* (Reinke & Berthold, 1879) and powdery scab (*Spongospora subterranea* f.sp. *subterranean* (Wallr.) Lagerh, 1892) (Rykbost *et al.*, 1995; Van der Waals *et al.*, 2013 & 2016). Potato soft rot/blackleg complex primarily caused by *Pectobacterium carotovorum* subsp. *brasiliense* (Nabhan *et al.*, 2012) is another disease reported to have increased in South African potato cultivations in recent years (Van der Waals *et al.*, 2016).

In terms of pests, insect pests that cause major concerns for South African potato producers include leaf miners (*Liriomyza trifolii* Burgess, 1880 and *Liriomyza huidobrensis* Blanchard, 1926) and potato tuber moth (*Phthorimaea operculella* Zeller, 1873 (Lepidoptera: Gelechiidae)) (PSA, 2017; Rondon, 2020; Yuan *et al.*, 2018). Plant-parasitic nematodes represent another pest that damages local potato crops (Jones *et al.*, 2017) and are discussed below.

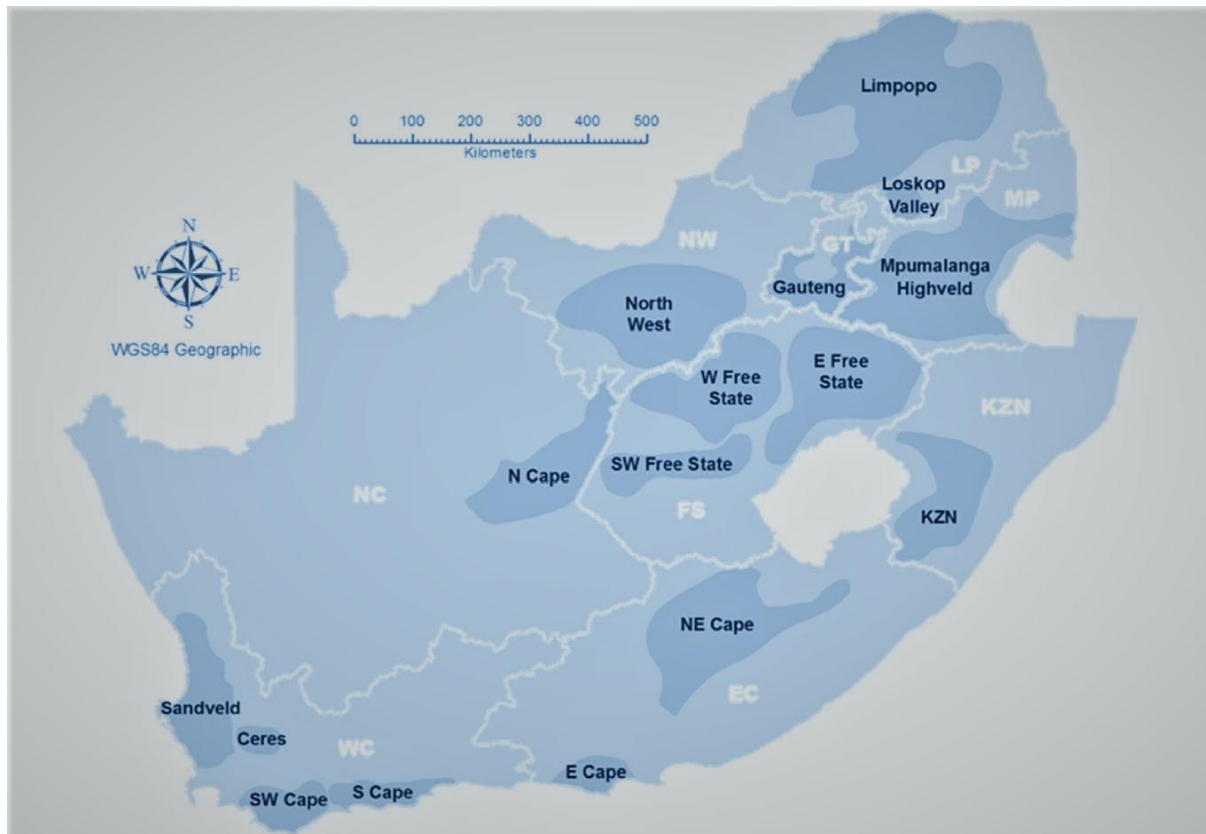


Figure 1.1: Map of South Africa showing the 16 potato growing regions in the country (approximate positions) (L. van Zyl, TerraGIS, South Africa, 2016).

### 1.3 Nematodes

Determining the presence and identity of target PPN is the first step towards nematode control (Marais *et al.*, 2017; Varandas *et al.*, 2020). Similarly, to determine the soil health status of a field, beneficial/non-parasitic nematodes (NPN) occurring in soils represent a group of the numerous biotic parameters used as bioindicators of soil health and need to be identified (Sanchez-Moreno & Ferris, 2018).

#### 1.3.1 Nematode sampling, extraction, counting and identification

To ensure that an accurate estimation of nematode populations is obtained from a potato field, it is very important that correct sampling of the soil and roots/other below-ground parts (e.g. tubers in the case of potato) of the intended crop is done. A general guideline here is to first map out

where in the site subsamples of soil and roots will be collected, from both healthy and symptomatic plants (or areas of good and poor growth) to get a representative picture of the field, using a random or systematic pattern (Coyne *et al.*, 2007). A soil sample should ideally be taken from 15 plants in a 20 cm deep hole for shallow-rooted crops (annual crops) and to a depth of 60 cm for deeper rooted crops (tree and perennial crops). Samples should be stored in cool conditions until they are used (Hallman & Subbotin, 2018).

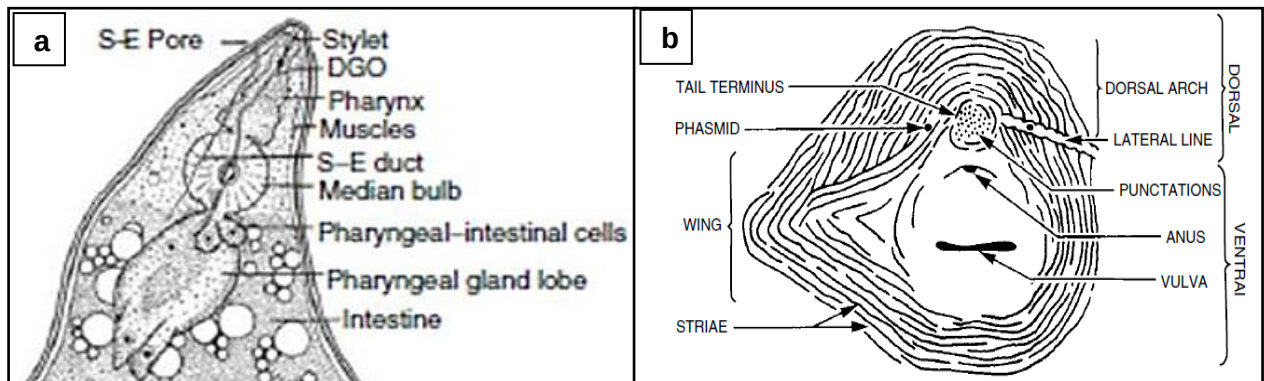
Different methods for extracting nematodes from soil and plant material exist and depend on the size, motility or specific gravity of nematodes (Dropkin, 1980a; Marais *et al.*, 2017). The adapted sodium hypochlorite (NaOCl) method developed by Riekert (1995) is used to extract RKN eggs and second-stage juveniles (J2) from roots and tubers, while the adapted sugar flotation method can be used to extract all life stages of RKN nematodes from roots and other plant tissues. To extract both active and inactive nematodes from the soil, the decanting and sieving method is used (Marais *et al.*, 2017). The extracted nematodes can then be counted using a dissecting or stereomicroscope and counting dish or be further identified using morphological keys of molecular techniques. According to Hassan *et al.* (2013), once nematodes have been extracted, determining the population density and correct identification follows. These are key facets to the use of proper management strategies which are crucial to successfully combat population build-ups of the target nematode pests. However, while implementing these strategies one should refrain from adversely impacting on beneficial nematodes (Hassan *et al.*, 2013; Varandas *et al.*, 2020).

Arguments concerning the replacement of morphological taxonomy with molecular techniques for identification have been increasingly voiced over the years, as molecular techniques have become more specialised and frequently utilised. Molecular techniques provide results in much shorter periods than morphological methods, can be automated and can help in the re-ordering and improvement of classification systems (Brito *et al.*, 2004; Dos Santos *et al.*, 2019). However, some information obtained from molecular techniques will not make any sense if the associated morphological characteristics of the nematode are not known (Dos Santos *et al.*, 2019).

#### **1.3.1.1 Morphological identification of plant-parasitic nematodes**

To identify a nematode to species level, it is critical that one has in-depth knowledge and vast experience of important characteristics that will indicate the species studied (Kleynhans *et al.*, 1996; Van Bezooijen, 2006). Morphological identification of PPN to species level is considered a highly specialised field of expertise, due to the numerous characteristics that must be studied (Collett *et al.*, 2021; Marais *et al.*, 2017). Some of these characteristics may go completely unnoticed to even the trained eye, emphasising the importance of well prepared and preserved specimens. Differentiating between PPN species of the same genus, such as species of RKN,

LN and GCN, using morphological characteristics observed in, for example, their oesophageal and perineal-pattern areas (for RKN see Figure 1.2 a & b), vulval cone areas and various other areas often prove to be confusing and inconclusive (Hu *et al.*, 2011). This is because nematodes are morphologically conservative and certain characteristics differ little within and between populations.



Figures 1.2: a. Oesophageal region of mature root-knot nematode female; b. Schematic illustration of the characteristics of a mature root-knot nematode female (Illustrations adapted from Karssen & Moens, 2006 and Rashidifard, 2019).

Characteristics of various RKN life stages (females, males and J2) that are used to identify a specimen to species level include, amongst others, the following: body shape and size; labial region; stylet, stylet cone and basal knobs' shape and size; a position of excretory pore from the anterior end; oesophageal lumen lining; length of dorsal gland opening behind stylet knob; perineal pattern characteristics (females); the position of phasmids and shape and length of spicules (males) (Rashidifard *et al.*, 2019).

Since this study mainly focussed on RKN, the following part of this literature review relating to PPN in particular mostly refers to this genus and in some cases to LN. Nonetheless, since beneficial nematodes were also focussed on for a small part of this study, this group is also mentioned due to some aspects related to their morphology and biology.

### 1.3.1.2 Molecular techniques used to identify plant-parasitic nematodes

Accurate and optimised molecular identification techniques have been developed for agriculturally important nematode species such as those listed as damaging to potato, as well as other PPN species affecting other crops, especially in morphologically similar genera or where cryptic species have occurred (Hallmann & Subbotin, 2018; Hunt *et al.*, 2018). The use of molecular techniques, specifically for RKN species identification, deoxyribonucleic acid (DNA) barcoding and taxonomy have been increasing and the technologies used are continuously undergoing improvement (Janssen *et al.*, 2017).

The DNA-based methodology has been used extensively during the past few decades for the identification of species of the economically most important RKN nematode genera that damage potato, but also for LN and GCN species (Collett *et al.*, 2021; Mao *et al.*, 2019; Orlando *et al.*, 2020). A concise reference to molecular techniques that are used and improved to optimally detect RKN species is listed in Table 1.1. These molecular techniques rely on polymerase chain reactions (PCR) to amplify DNA segments, except for recombinase polymerase amplification.

#### **1.3.1.3 Morphological and molecular identification of beneficial soil nematodes**

Successful identification of beneficial soil nematodes uses the same techniques as those employed in the identification of PPN (see Paragraphs 1.3.1.1 and 1.3.1.2). The general size and behaviour of beneficial soil nematodes differ substantially from that of the PPN. Some beneficial soil nematodes move rapidly, while others, such as the order Dorylaimida, tend to be much larger than PPN. The mouthparts of beneficial soil nematodes are used to categorise them into the different trophic groups, before attempting to identify the family, genus and species. The differences in the mouthparts of the different trophic groups are depicted in Figure 1.3, although this is a considerably basic depiction of the mouthparts and variation exists within each trophic group, between the various genera. The shape, length and characteristics of the tail region may also be used to aid in identifying beneficial soil nematodes. Each genus has its distinct tail characteristics, which may even vary between males, females and juveniles of the same genus (Heyns, 1971 & 1982).

Molecular techniques used to identify beneficial nematode species include many of the techniques discussed for PPN, as listed in Table 1.1, utilising mitochondrial DNA (mtDNA) and ribosomal DNA (rDNA), but most studies still use well preserved samples and microscopy. Great progress had been made to identify beneficial nematodes, from a relatively limited number of studies conducted to date in South African natural veld and agricultural fields, using these methods in the past decade (Girgan *et al.*, 2018 & 2020; Swart *et al.*, 2020).

Table 1.1: Molecular methods used to successfully identify root-knot nematode (RKN) species.

| Method                                                    | Description                                                                                                                                                                                                                 | Root-knot nematode (RKN) species                                                                                          | Advantage                                                                                                                                          | Limitations                                                                                                                                            | Reference                                                                                                               |
|-----------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------|
| <b>Sequence characterised amplified region (SCAR-PCR)</b> | Species-specific primers designed using SCAR) markers to identify an organism.                                                                                                                                              | Most RKN species, especially for differentiating some such as <i>M. enterolobii</i> from morphologically similar species. | Fast. Multiple species identified in one PCR test. Allows identification of species in a population/community with small sequence differentiation. | Number of primer pairs and gel electrophoresis (GE) bands that can be used in a single reaction without obtaining false-positive results.              | Collett <i>et al.</i> (2021); Dos Santos <i>et al.</i> (2019); Hallmann & Subbotin (2018); Tigano <i>et al.</i> (2010). |
| <b>Restriction fragment length polymorphism (RFLP)</b>    | Determines distances and sites at which specific restriction endonucleases cleave DNA strands. PCR & GE generates band patterns. Differences and similarities in patterns produced are used to distinguish between species. | Most described RKN species                                                                                                | Comparing new RFLP profiles to existing ones is a quick method of identification.                                                                  | Very time consuming and labour intensive. Cannot be used for mixed communities.                                                                        | Da Cunha <i>et al.</i> (2018); Hallman & Subbotin (2018); Janssen <i>et al.</i> (2016).                                 |
| <b>Amplified fragment length polymorphism (AFLP)</b>      | Based on the selective amplification of genomic restriction fragments.                                                                                                                                                      | Good for studying the inter- and intraspecific genetic variation of RKN.                                                  | Results are highly reproducible.                                                                                                                   | Labour intensive.                                                                                                                                      | Hallmann & Subbotin (2018); Tigano <i>et al.</i> (2010).                                                                |
| <b>Recombinase polymerase amplification (RPA)</b>         | RPA uses highly efficient displacement polymerase to amplify a few copies of target nucleic acid rapidly at a constant temperature.                                                                                         | <i>M. enterolobii</i>                                                                                                     | Efficient & fast alternative for PCR. Can be carried out in real time. Versatile.                                                                  | Very little supporting literature. RPA specific primers have only been developed for <i>M. enterolobii</i> thus far.                                   | Ju <i>et al.</i> (2019); Subbotin (2019)                                                                                |
| <b>Identification using mitochondrial DNA (mtDNA)</b>     |                                                                                                                                                                                                                             |                                                                                                                           |                                                                                                                                                    |                                                                                                                                                        |                                                                                                                         |
| <b>Cytochrome oxidase subunit 1 (COI)</b>                 | Using the COI gene region on the 16S rRNA region of mtDNA as a barcode to amplify nematode DNA with PCR. Species are then identified with DNA sequencing.                                                                   | Most RKN species.                                                                                                         | Primer sets are usually genus specific. Aid in the discovery of previously undescribed species.                                                    | Limited scope for use in taxonomy. Requires well-curated sequence databases to be accurate. Combination with other techniques is crucial for accuracy. | Moore <i>et al.</i> (2020); Pretorius (2018); Powers <i>et al.</i> (2018); Subbotin (2019).                             |
| <b>Cytochrome oxidase subunit 2 (COII)</b>                | Using the COII gene region on the 16S rRNA region of mtDNA as a barcode to amplify nematode DNA with PCR                                                                                                                    | Most RKN species                                                                                                          | Overcomes limitations of rDNA techniques, due to mtDNA genes having uniparental inheritance and high mutation rates.                               | COII regions have insufficient variability to reliably differentiate between <i>M. incognita</i> , <i>M. javanica</i> and <i>M. arenaria</i> .         | Janssen <i>et al.</i> (2016); Moore <i>et al.</i> (2020); Pretorius (2018); Rutter <i>et al.</i> (2019).                |

Table 1.1: Molecular methods used to successfully identify root-knot nematode (RKN) species (continued).

| Method                                                                                                                      | Description                                                                                                                           | Root-knot nematode (RKN) species                                                                               | Advantage                                                                                                                             | Limitations                                                                                                                                                                                             | Reference                                                                                                        |
|-----------------------------------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------|
| <b>Nicotinamide adenine dinucleotide (NADH) dehydrogenase subunit 5</b>                                                     | Using the NADH5 gene region of the 16S rRNA as a barcode to amplify nematode DNA with PCR.                                            | Most RKN species                                                                                               | Can reliably identify major lineages of tropical/thermophilic RKN species. Better suited for use in sequencing than COI & COII genes. | Needs to be combined with other molecular and morphological techniques to ensure accuracy.                                                                                                              | Chitambo <i>et al.</i> (2019); Janssen <i>et al.</i> (2016); Pretorius (2018); Rashidifard <i>et al.</i> (2019). |
| <b>Identification using ribosomal DNA (rDNA):</b>                                                                           |                                                                                                                                       |                                                                                                                |                                                                                                                                       |                                                                                                                                                                                                         |                                                                                                                  |
| <b>Spacer Regions: Internal Transcribed spacer (ITS). External transcribed spacer (ETS). Intergenic region spacer (IGS)</b> | Sequencing of these segments to identify species.                                                                                     | Most RKN species, e.g., IGS used to distinguish <i>M. chitwoodi</i> & <i>M. fallax</i> from other RKN species. | Several protocols are available for nematode identification.                                                                          | <i>M. arenaria</i> , <i>M. incognita</i> & <i>M. javanica</i> have very little differentiation in their ITS regions. Time consuming. Multiple types of ITS within a species/individual limits accuracy. | Da Cunha <i>et al.</i> (2018); Rashidifard <i>et al.</i> (2018 & 2019).                                          |
| <b>D2-D3</b>                                                                                                                | Sequencing of the D2-D3 segment of 28S ribosomal rDNA                                                                                 | All described RKN species.                                                                                     | Requires a single individual for DNA extraction. Used to design and verify results from other techniques e.g., SCAR-PCR.              | Time consuming. Requires a well-curated database for true accuracy.                                                                                                                                     | Ghaderi <i>et al.</i> (2014); Rashidifard <i>et al.</i> (2019); Rutter <i>et al.</i> (2019);                     |
| <b>Genotyping sequencing (GBS) by</b>                                                                                       | Next-generation sequencing (NGS) of genomic fragments obtained by specific restriction enzymes followed by a bioinformatics pipeline. | Any. Has shown to be accurate in characterising nematodes species when no genome information was available.    | Simple protocol. Reduces sequencing costs, while obtaining genome-wide information. No reference sequence limits.                     | Very few limitations. Methods and quality of protocols and results are continuously improving.                                                                                                          | He <i>et al.</i> (2014); Rashidifard <i>et al.</i> (2018)                                                        |

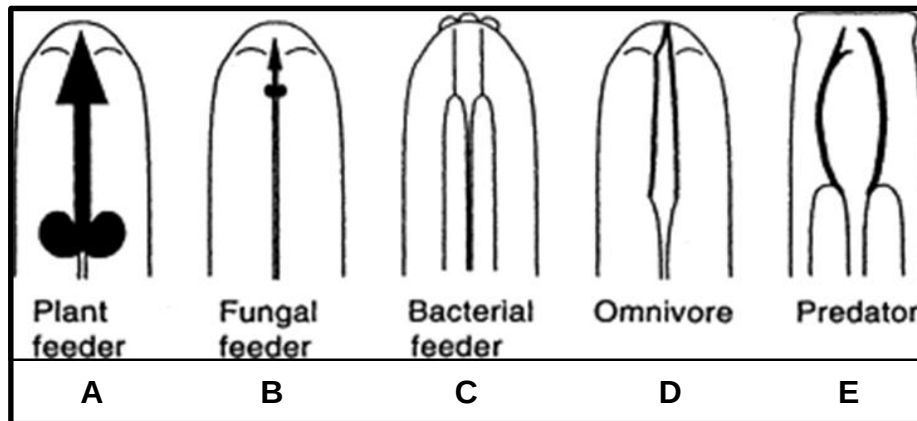


Figure 1.3: Schematic illustration of different mouthparts for the different trophic groups of nematodes on which focus is also placed in this study. A. Plant-parasitic nematode; B-E. Beneficial nematodes (Bouwman *et al.*, 2005).

### 1.3.2 Basic nematode morphology and physiology

Nematodes are commonly known as roundworms with a thread-like body shape. The word nematode is derived from the Greek words 'nemas', meaning thread and 'eidos', meaning form (Decraemer & Hunt, 2006). Nematodes have been found in nearly every environment imaginable, but they require some amount of moisture to facilitate movement and survival, as they are aquatic and live in the water film surrounding soil particles (Gaugler & Bilgrami, 2004; Kleynhans *et al.*, 1996). The general structure consists of the body wall, pseudocoelom, head and tail region, digestive tract and reproductive system. The body wall is composed of an epidermis and cuticle (Decraemer & Hunt, 2006).

Plant-parasitic nematodes can easily be distinguished from others by the presence of a robust stylet, situated in the mouth cavity that is usually connected to basal stylet knobs (Figure 1.3A). The stylet may be either a stomatostyle or odontostyle, which is hollow, or an onchiostyle, which is solid (Van den Berg *et al.*, 2017). Beneficial soil nematodes on the other hand do not possess stylets, except for those that feed on fungi (Figure 1.3B) and those that feed on other micro-organisms (Figure 1.3D) (APS, 2021; McSorley, 2019). This group of nematodes has a range of feeding mechanisms that are often used to divide them into five basic trophic levels for bioindicator purposes, as shown in Table 1.2.

Table 1.2: Feeding habits of plant-feeding and beneficial soil nematodes (Nemaplex, 2021; Yeates *et al.*, 1993).

| Feeding habit & feeding code                                                 | Trophic group | Feeding apparatus                                        | Example                                                                                                       |
|------------------------------------------------------------------------------|---------------|----------------------------------------------------------|---------------------------------------------------------------------------------------------------------------|
| <b>Plant feeding (H)</b>                                                     | Herbivore     | Stylet (piercing)                                        | <i>Meloidogyne</i> , <i>Pratylenchus</i> ,<br><i>Heterodera</i> , <i>Criconea</i> ,<br><i>Helicotylenchus</i> |
| <b>Fungal (F): hyphal feeding</b>                                            | Fungivore     | Stylet (piercing)                                        | <i>Aphelenchus</i> , <i>Filenchus</i> ,<br><i>Dorylaimoides</i> , <i>Funaria</i> ,<br><i>Leptonchus</i>       |
| <b>Bacterial feeding (B)</b>                                                 | Bacterivores  | Buccal cavity (engulfing/sucking)                        | <i>Cephalobus</i> , <i>Rhabditis</i>                                                                          |
| <b>Predation (P): specialist predators of nematodes</b>                      | Predators     | Buccal cavity with one or more teeth (cutting/engulfing) | <i>Mononchus</i> , <i>Sphaerolaimus</i> ,<br><i>Discolaimus</i>                                               |
| <b>Omnivory (O): general predators of nematodes and other soil organisms</b> | Omnivores     | Varied (stylet or mural tooth)                           | <i>Baladorylaimus</i> ,<br><i>Chrysonemoides</i> , <i>Actinolaimus</i>                                        |

### 1.3.2.1 Basic life cycle of nematodes

The nematode life cycle has an egg stage which is generally followed by four juvenile stages and finally the adult male and female stages (Decraemer & Hunt, 2006; Ingham & Jensen, 2021). The life cycle of PPN associated with potato is elaborated on in paragraphs 1.3.3.1 and 1.3.3.2.

Beneficial soil nematodes have similar life stages as those described for PPN. The juveniles closely resemble the adult females, although they lack the reproductive systems and are smaller than the adults (Decraemer & Hunt, 2006). The life cycle of beneficial soil nematodes tends to differ between species and is linked to their respective survival strategies and functional roles in the soil food web, as discussed in par. 1.3.4.

### 1.3.2.2 Reproduction

Nematodes can reproduce either sexually or asexually depending on the species (Decraemer & Hunt, 2006). The female reproductive system varies from genus to genus and nomenclature describing this depends on the position, direction and number of uteri (Van den Berg *et al.*, 2017). Root-knot nematode females are diovarial and prodelphic, meaning their reproductive system consists of two ovaries and the uterus is directed anteriorly (vulva located at the posterior of a mature, swollen female). Eggs are deposited as egg masses (on or below the surface of the infected below-ground plant part) which are protected by a gelatinous matrix, excreted from six rectal glands (Nemaplex, 2021; Rashidifard, 2019). Opposed to RKN females, the LN differs from RKN regarding their reproductive systems, as the females of LN does not become swollen. The LN females have a single ovary (monovarial) with the posterior branch generally reduced into a post-uterine sac and are prodelphic. Eggs are deposited in the roots or soil and are not contained in a matrix or cyst (Nemaplex, 2021; Singh *et al.*, 2018).

### 1.3.2.3 Survival Strategies

When a host plant is not available, or environmental conditions are not favourable, PPN have several survival strategies or dormant stages to use until conditions change and become favourable for hatching of the infectious life stages. Dormancy refers to a state of lowered metabolic activity (Hunt *et al.*, 2018; Oka, 2019; Perry *et al.*, 2006). Anhydrobiosis, a process where the nematode loses almost all its body water content and stops all metabolic activity (desiccation-induced dormancy), is a survival strategy used by many PPN, including most LN species (Oka, 2019; Ribeiro *et al.*, 2020).

Root-knot nematodes, on the other hand, tend to survive as egg masses, eggs in obligate quiescence (needing plant root exudates to hatch) or by the J2 migrating deeper into the soil substrate. These strategies are mostly used in colder climates to avoid freezing. Oka (2019) however suggests that J2 of the RKN species *Meloidogyne javanica* (Treub, 1885; Chitwood, 1949) survive in dry conditions through anhydrobiosis or in the drought-resistant structure of egg masses.

### 1.3.3 Plant-parasitic nematodes' damage potential to potato

The total crop loss (per annum) in South Africa, due to infection by PPN, has been valued at over ZAR1.9 billion for both horticultural and agricultural crops, while globally, the annual crop loss due to PPN has been estimated to approximately US\$157 billion (Sikandar *et al.*, 2020; Singh *et al.*, 2015; Steyn *et al.*, 2014).

In South Africa, except for RKN, LN and the GCN damaging potato, another 450 genera have been found, with 95 (21%) of these linked to potato production areas according to data assimilated by the South African Plant-Parasitic Nematode Survey (SAPPNS) and the National Collection of Nematodes (NCN) (Marais *et al.*, 2015). From this data, six *Meloidogyne* spp., 12 *Pratylenchus* spp. and *G. rostochiensis* contribute to the challenges facing South African potato producers (Jones *et al.*, 2017; Marais *et al.*, 2015; Varandas *et al.*, 2020). Although species of other cyst genera, namely *Punctodera* and *Heterodera* (Schmidt, 1871), have also been associated with local potato production, no information is available on their pathogenicity and/or whether they are pests of the crop. Other PPN genera also associated with potato are *Helicotylenchus* Steiner 1945, *Nanidorus* Siddiqi 1974, *Paratrichodorus* Siddiqi 1974, *Telotylenchus* Siddiqi 1960 and more (see Paragraph 1.3.9) (Jones *et al.*, 2017; Marais *et al.*, 2015; Youssef, 2013). Stubby-root nematodes, from the family *Trichodoridae* Thorne, 1935, that infect and damage potatoes globally as well as locally are *Nanidorus*, *Paratrichodorus* and *Trichodorus* (Jones *et al.*, 2017; Kleynhans *et al.*, 1996; Lima *et al.*, 2018). These nematodes are ectoparasitic root-tip feeders and can cause

severe damage to crops and can in some cases be considered as virus vectors (Jones *et al.*, 2017; Kleynhans *et al.*, 1996; Lima *et al.*, 2018).

A wide variety of other nematode genera also infect local potato crops, but to a lesser extent and these genera differ per growing region. Ring nematodes (*Criconeematidae* Taylor, 1936) seem to mainly occur in the Eastern Cape, KwaZulu-Natal, North West, Gauteng and Mpumalanga provinces of South Africa. Spiral nematodes (*Helicotylenchus*) also occur mainly in these areas, but have a prominent presence in the Sandveld and Northern Cape areas. Stunt nematodes (*Geocenamus* Throne & Malek, 1968, *Telotylenchus* and *Tylenchorhynchus* (Cobb, 1913)) mostly occur in the Sandveld, Gauteng, Loskop Valley and Mpumalanga areas (Marais *et al.*, 2015). Generally, the physical and chemical changes caused to potato crops by PPN include deformed tubers, enhanced chitinase activity, reduced tuber formation, weakened plants, chlorosis and necrosis (Figure. 1.4) (Coyne *et al.*, 2018; Singh *et al.*, 2015; Youssef, 2013). Plant-parasitic nematodes can be divided into seven feeding/trophic groups (Table 1.3), depending on where and how they interact with their host plant.



Figure 1.4: Root-knot nematode infection causing foliar symptoms on potato (Photo: Abawi & Gugino, 2014).

Table 1.3: The feeding/trophic groups of plant-parasitic nematodes (Decraemer & Hunt, 2006; Skantar, 2020; Wheeler & Crow, 2020).

| Mode of feeding                | Description                                                                                                                                                       | Example                                                                | Damage                                                                                                    | Association with potato |
|--------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------|-------------------------|
| <b>Ectoparasites</b>           | Remain in the soil and feed off the plant-host by using the stylet to penetrate plant cells.                                                                      | Species of Criconematidae, Longidoridae, Paratrichodoridae.            | Disruption of normal meristem functions.                                                                  | Yes                     |
| <b>Semi-endoparasites</b>      | Partially enter the plant tissue with part of their bodies being in the soil.                                                                                     | Species of <i>Rotylenchulus</i> , <i>Tylenchulus</i> .                 | Reduced crop size, reduced tree vigour due to damaged feeder roots.                                       | Yes                     |
| <b>Migratory endoparasites</b> | Move through root tissues and destructively feed on the plant cells and could be associated with secondary disease infections.                                    | Species of <i>Pratylenchus</i> , <i>Radopholus</i>                     | Cortex tissues. Creates sites for secondary infection by other pathogens.                                 | Yes                     |
| <b>Sedentary endoparasites</b> | The infective second-stage juvenile (J2) generally infests root tips/other below-ground parts into the vascular tissues and establishes a permanent feeding site. | Species of <i>Globodera</i> , <i>Heterodera</i> , <i>Meloidogyne</i> . | Hypertrophy (cell enlargement) and hyperplasia (galling) in root systems, reducing normal root functions. | Yes                     |
| <b>Stem and bulb nematodes</b> | Feed on stems and bulbs of plants and migrate up the plant in water films.                                                                                        | Species of <i>Ditylenchus</i> .                                        | Cortex tissues and sloughing. Creates sites for secondary infection by other pathogens.                   | Yes                     |
| <b>Seed-gall nematodes</b>     | Form galls on the seed of plants, such as grasses                                                                                                                 | Species of <i>Anguina</i> , <i>Subanguina</i> .                        | Galling, deformation and reduced quality of seed.                                                         | No                      |
| <b>Foliar nematodes</b>        | Utilise water films to move up from the stems to the leaves of a plant to feed.                                                                                   | Species of <i>Aphelenchoides</i> , <i>Bursaphelenchus</i>              | Patchy browning and necrosis of leaves.                                                                   | No                      |

### 1.3.3.1 Root-knot nematodes

The most common nematode problem perceived and widely observed by producers and generally considered the most destructive to potato in South Africa is RKN (Jones *et al.*, 2017). This genus is polyphagous, infecting a wide range of agricultural crops, non-host crops (e.g., natural veld and weeds), causing devastating losses (Coyne *et al.*, 2018; Onkendi *et al.*, 2014; Varandas *et al.*, 2020). From the approximately 100 RKN species described, only seven have been associated with potato (Niere & Karakuri, 2018). The most prominent RKN species associated with potato in South Africa are *Meloidogyne incognita* (Kofoid & White, 1919) Chitwood, 1949 and *M. javanica* (Jones *et al.*, 2017). Other species that have been listed to infect local potato crops include *Meloidogyne arenaria* (Neal, 1889) Chitwood, 1949; *Meloidogyne enterolobii* Yang & Eisenback, 1983; *Meloidogyne chitwoodi* Golden, O'Bannon, Santo & Finley, 1980; and *Meloidogyne hapla* Chitwood, 1949 (Jones *et al.*, 2017; Marais *et al.*, 2015; Niere & Karakuri, 2018).

### Life cycle

Figure 1.5 illustrates the life cycle of a RKN that is explained here. Typically, for RKN, the first-stage juvenile (J1) occurs in the egg and undergoes its first moult in the egg after which it emerges as the motile J2 (Decraemer & Hunt, 2006; Hunt *et al.*, 2018; Kleynhans, 1999). The infectious stage of this specific genus is the J2 that infects the roots/other below-ground parts of a host plant

and develops to a swollen J2, then into a swollen third-stage juvenile (J3) followed by a swollen fourth-stage juvenile (J4). The latter two stages do not have stylets and do not feed. The J4 goes through a final moult and becomes either an adult motile male or swollen female. Both males and females exhibit a stylet, but only the female actively feeds (Decraemer & Hunt, 2006; Hunt *et al.*, 2018).

Root-knot nematode species are known as sedentary endoparasitic nematodes as the swollen adult females reside inside the galls, either singularly or in multitudes and produce egg sacs that may protrude to the exterior of the gall (Jones *et al.*, 2013). Infection of a host plant occurs when a J2 invades the root/other below-ground plant parts near or at the root tip. The J2 then migrates inside the root to an area of actively differentiating vascular tissues (cell elongation). As the J2 feeds, several giant cells develop around its head and eventually engulf the nematode as it moults and matures into a swollen female, subsequently forming the galls. The life cycle of *Meloidogyne* spp. takes approximately 21 days, depending on its host and the surrounding soil temperature (Dropkin, 1980a; Jones *et al.*, 2017; Khan *et al.*, 2017).

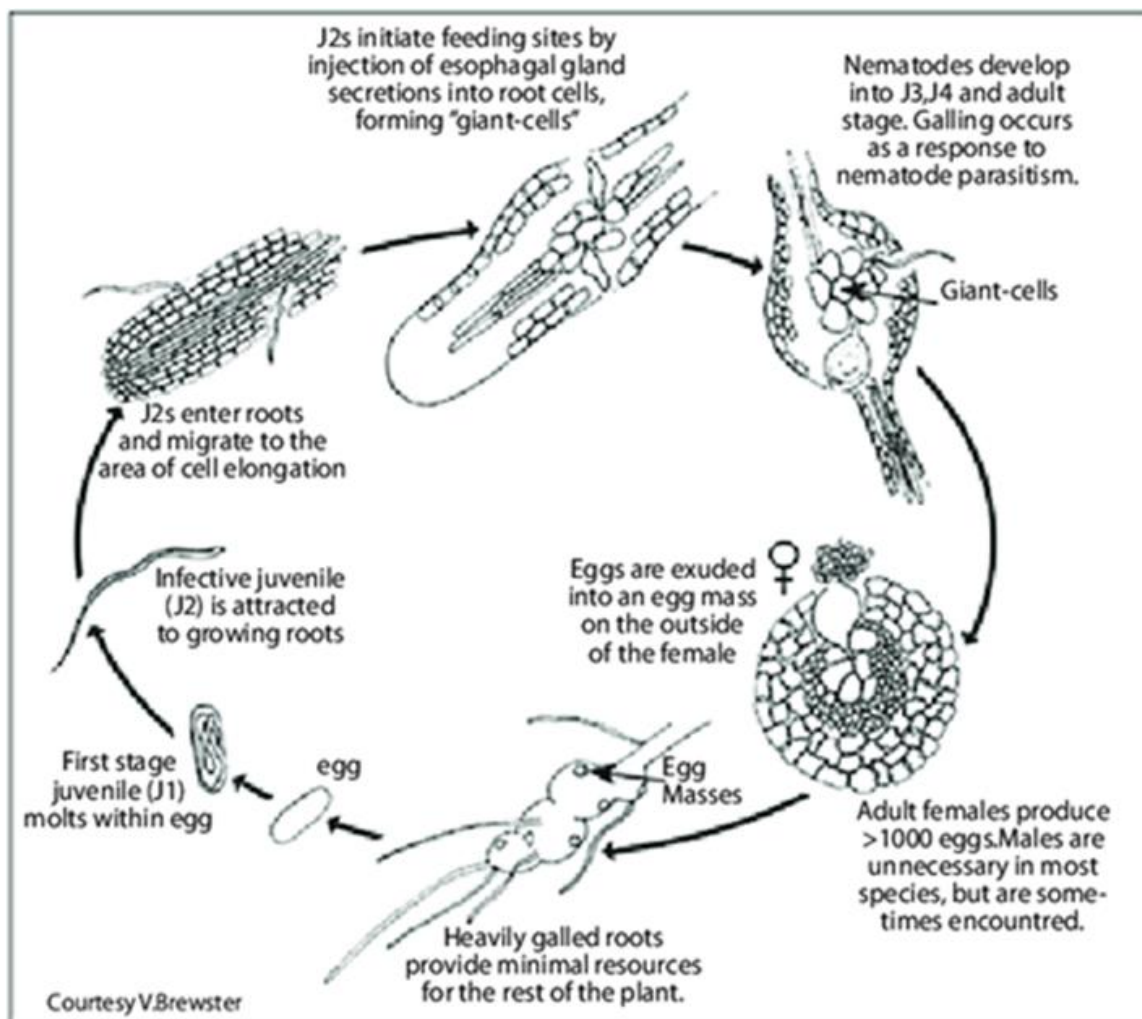
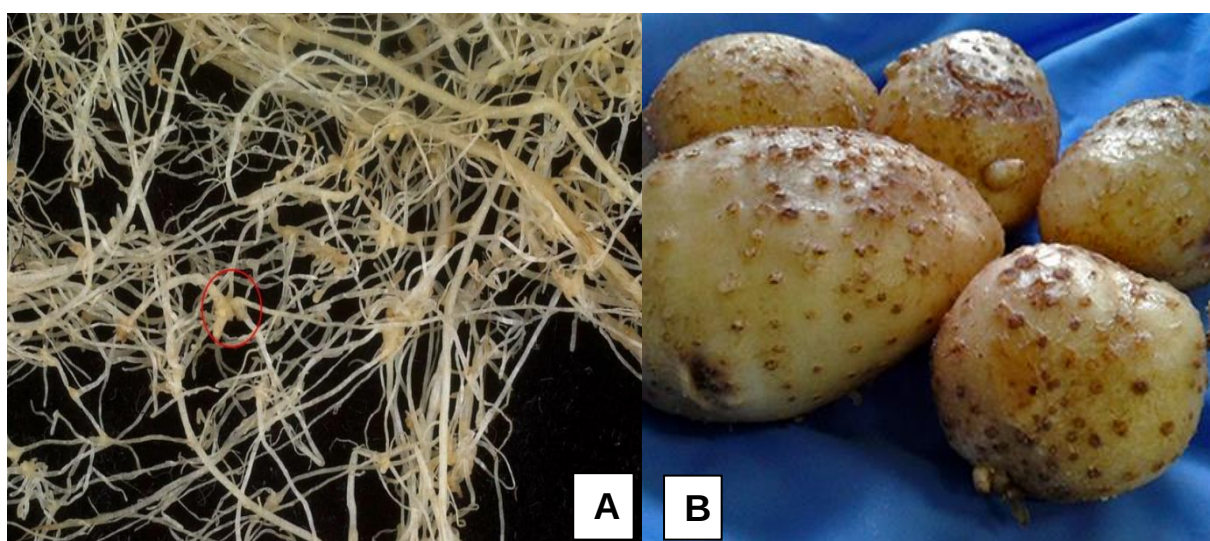


Figure 1.5: Life cycle of a root-knot nematode (Khan *et al.*, 2017).

### **Damage and symptoms**

Above-ground symptoms associated with RKN are typically that of plants with poor or unhealthy root systems or can be confused with other factors such as nutrient or water deficiency, poor soil fertility or other diseases but is usually seen as patches where plants do not grow optimally in a field (Jones *et al.*, 2017; Niere & Karakuri, 2018).

A below-ground symptom associated with RKN infestation is typically the formation of galls on the roots and tubers of potato plants. These galls may appear as small bumps on the roots and tubers or in severe cases, these plant parts can be completely deformed with large growths distorting their natural shape (Niere & Karakuri, 2018; Jones *et al.*, 2017) (Figure 1.6A & B).



Figures 1.6: A) Root-knot nematode damaged potato roots reflecting typical gall formation (Photo: Abawi & Gugino, 2014); B) Root-knot nematode damage on potato tubers (<https://www.arc.agric.za/arc-vopi/Pages/Crop%20Protection/Nematodes.aspx>).

If a population of RKN is present in the soil when tuber initiation starts, viable J2 will readily infest the developing tubers. Depending on the growing season and storage methods utilised (e.g. soil storage), a substantial portion of the tubers could become damaged, resulting in a reduced marketable yield (Jones *et al.*, 2017).

### **Interactions with other soil micro-organisms**

As RKN feed and reproduce, the tissues of the roots and tubers are exposed to other soilborne pathogens, such as fungi and bacteria creating opportunities for secondary infection which in turn can significantly increase crop and yield losses (Ali *et al.*, 2020; Ireri *et al.*, 2019). A limited number of studies have been done in the last decade on the interactions existing between RKN and potato specific diseases. Root-knot nematodes have been associated with an increase in severity of

potato diseases, such as bacterial wilt (*Ralstonia solanacearum* (Smith 1896) Yabuuchi et al. 1996) and soft rot (Enterobacteriaceae Rahn, 1937). The increased pathogenicity of these diseases in such interactions is attributed to the ability of RKN to weaken disease-resistance mechanisms of the potato plants (Bekhiet et al., 2010; Mongae, 2013).

### 1.3.3.2 Lesion nematodes

The LN are polyphagous, migratory endoparasites (opposed to RKN that are sedentary endoparasites) that feed by entering and exiting roots and/or tubers freely and migrate between roots and/or tubers and soil particles (Lima et al., 2018; Vos et al., 2012). They cause necrotic lesions in the cortex tissues of infected roots and tubers, resulting from their feeding. This genus is considered the second most economically damaging PPN genus in agriculture globally (Lima et al., 2018; Vos et al., 2012). Species from this genus can be found globally in tropical and subtropical regions and have a wide host range, like RKN (Fanning et al., 2018; Jones et al., 2017).

At least 15 species of LN have been reported to infect potato worldwide (Lima et al., 2018). In South Africa, 12 LN species have been found infecting potato and are listed in Table 1.4 with the most predominant being *Pratylenchus brachyurus* (Godfrey, 1929) Filipjev & Schuurmans Stekhoven, 1941 (Jones et al., 2017; Marais et al., 2015).

Table 1.4: Lesion nematodes associated with potato in South Africa (Marais et al., 2015).

| Species                                                                                | Species                                                     |
|----------------------------------------------------------------------------------------|-------------------------------------------------------------|
| <i>Pratylenchus brachyurus</i> (Godfrey, 1929) Filipjev and Schuurmans Stekhoven, 1941 | <i>Pratylenchus pratensis</i> (De Man, 1880) Filipjev, 1936 |
| <i>Pratylenchus delattrei</i> Luc, 1958                                                | <i>Pratylenchus scribneri</i> Steiner, 1943                 |
| <i>Pratylenchus flakkensis</i> Seinhorst, 1968                                         | <i>Pratylenchus teres</i> Khan and Singh, 1975              |
| <i>Pratylenchus hexincisus</i> Taylor and Jenkins, 1957                                | <i>Pratylenchus thornei</i> Sher and Allen, 1953            |
| <i>Pratylenchus neglectus</i> (Rensch, 1924) Filipjev and Schuurmans Stekhoven, 1941   | <i>Pratylenchus vulnus</i> Allen and Jensen, 1951           |
| <i>Pratylenchus penetrans</i> (Cobb, 1917) Filipjev and Schuurmans Stekhoven, 1941     | <i>Pratylenchus zaeae</i> Graham, 1951                      |

### Life cycle

Unlike RKN where only the J2 are infective, all the hatched life stages of LN species (Figure 1.7) can move through root tissues and feed on cortical cells (Stirling et al., 2002; Vos et al., 2021). Lesion nematodes can produce several generations in a single crop cycle if soil and climatic conditions are favourable (Kleynhans et al., 1996; Lima et al., 2018). At different temperatures, the time span of a single life cycle of a LN varies greatly, from approximately 31 days at 30 °C to 86 days at 15 °C (Mamiya, 1971).

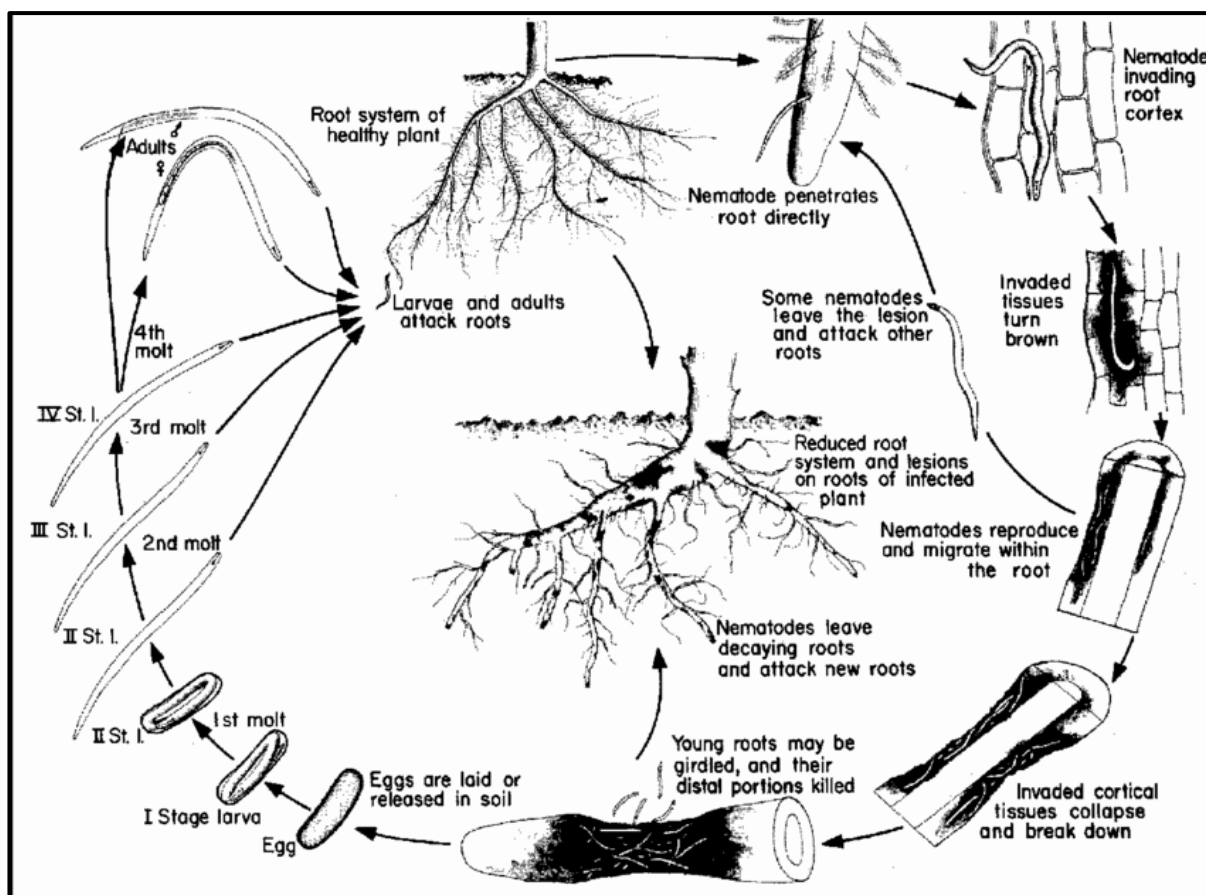


Figure 1.7: Disease cycle of a lesion nematode (Adapted from Agrios, 2005).

### Damage and symptoms

Lesion nematodes have a wide host range, including many agriculturally important crops, but also a variety of weed species (Fanning *et al.*, 2018; Jones *et al.* 2017; Vos *et al.*, 2012). Monocropping, or successive planting of susceptible host crops or even weeds between plantings can lead to the population build-up of LN in a field. Life stages from the J2 stage up to the adults of this genus feed by inserting its stylet into the cortex of the potato root and/or tuber. The feeding area forms a necrotic lesion on the roots and tubers that can be visible to the naked eye (Kleynhans *et al.*, 1996; Lima *et al.*, 2018). In potato, above-ground symptoms are remarkably similar to that of RKN. Lesion nematodes feeding on roots and tubers, especially in large numbers, can lead to above-ground symptoms such as crop stunting, wilting, delayed flowering and necrosis (Fig. 1.8) (Jones *et al.*, 2017). Infected crops will generally have poor growth seen in patches throughout the field (Lima *et al.*, 2018).



Figure 1.8: Above-ground symptoms of lesion nematode infestation in a potato field (<http://www.omafra.gov.on.ca/IPM/english/potatoes/nematode/nematode.html>).

Symptoms on the roots are usually similar to those seen in other affected crops. Symptoms on the tubers, however, include brown (from light in colour to near black) or greyish lesions (Jones *et al.*, 2017; Lima *et al.*, 2018). The lesions generally have raised centres surrounded by slight depressions. Heavily infested tubers sometimes develop a crust as the lesions coalesce. Although lesion nematode infection only affects the surface of the tubers, infected tubers have a substantially lower storage life than non-infected tubers (Jones *et al.*, 2017; Lima *et al.*, 2018) (Figure 1.9).

Damage by lesion nematodes also acts as a pathway for secondary infection by other soil-borne pathogens, sometimes obscuring the evidence of nematode feeding (Bjorsell *et al.*, 2017; Jones *et al.*, 2017; Singh *et al.*, 2015). This makes accurate estimating of crop losses due to lesion nematodes difficult but yield losses of up to 50% have been reported in Norway (Holgado *et al.* 2009) and Brazil (Lima *et al.*, 2018), depending on population densities as well as climatic and soil conditions (Holgado *et al.*, 2009; Jones *et al.*, 2017; Lima *et al.*, 2018). For South Africa, LN causes economically severe damage, although estimated costs or percentage crop losses have not been established. In 2018, it was estimated that the total nematode damage, caused by all PPN on potato and not only LN, contributed to 6% of the number of potatoes downgraded in quality on the market (PSA, 2021).



Figure 1.9: Cross-lesions on potato tubers from plants inoculated with 2000 specimens of *Pratylenchus penetrans* per pot in a glasshouse study (Adapted from Holgado *et al.*, 2009).

### ***Interactions with other micro-organisms***

Other soilborne pathogens interacting with LN are *Fusarium* and *Phytophthora* spp., *Rhizoctonia solani* and the fungus *Verticillium* causing *Verticillium*-wilt in potato (Edin *et al.*, 2019; Jones *et al.*, 2017).

Since RKN and LN were the predominant species found in the experimental site where this study was conducted, reference was made to their life cycles, damage and symptoms caused to potato and interactions with other organisms. Although GCN also causes damage to potato in South Africa, it was not present in the experimental site where this study was conducted and therefore, no reference in this regard was given to this species.

### **1.3.4 Beneficial nematodes and their use as bio-indicators of soil health**

Although PPN in agricultural terms are the most known and studied nematodes, they only constitute a small group of soil nematodes. Beneficial soil nematodes that occur in natural ecosystems in South Africa, in particular, have not been studied as in-depth as PPN, although this is slowly changing (Du Preez *et al.*, 2017; Girgan *et al.*, 2020; Swart *et al.*, 2017). These nematodes occur across the globe in every ecosystem, be it soil, freshwater sources, estuaries or caves (Girgan *et al.*, 2020; Liu *et al.*, 2019; Ugarte *et al.*, 2013). In this study, the focus was only on beneficial terrestrial nematodes that are used as bio-indicators in agricultural cropping systems.

#### **1.3.4.1 Soil health and soil quality indices**

A healthy soil was defined by Doran and Safley (1997) as “the continued capacity of soil to function as a vital living system, within the ecosystem and land-use boundaries, to sustain biological productivity, promote the quality of air and water environments and maintain plant,

animal and human health”. This concept refers to the physical, chemical and biological aspects of soil and how these affect the ability of the soil to facilitate plant growth and other environmental functions (Cardoso *et al.*, 2013; Doran & Safley, 1997; Munoz-Rojas, 2018).

Soil health parameters are also referred to as soil quality indexes (SQI) and are listed in Figure 1.10 which depicts the link between SQI and critical soil ecosystem processes, including water and climate regulation, carbon and nutrient cycling, soil microbe and flora diversity as well as land stability (Cardoso *et al.*, 2013; Munoz-Rojas, 2018). Figure 1.11 indicates the different components considered when studying soil health.

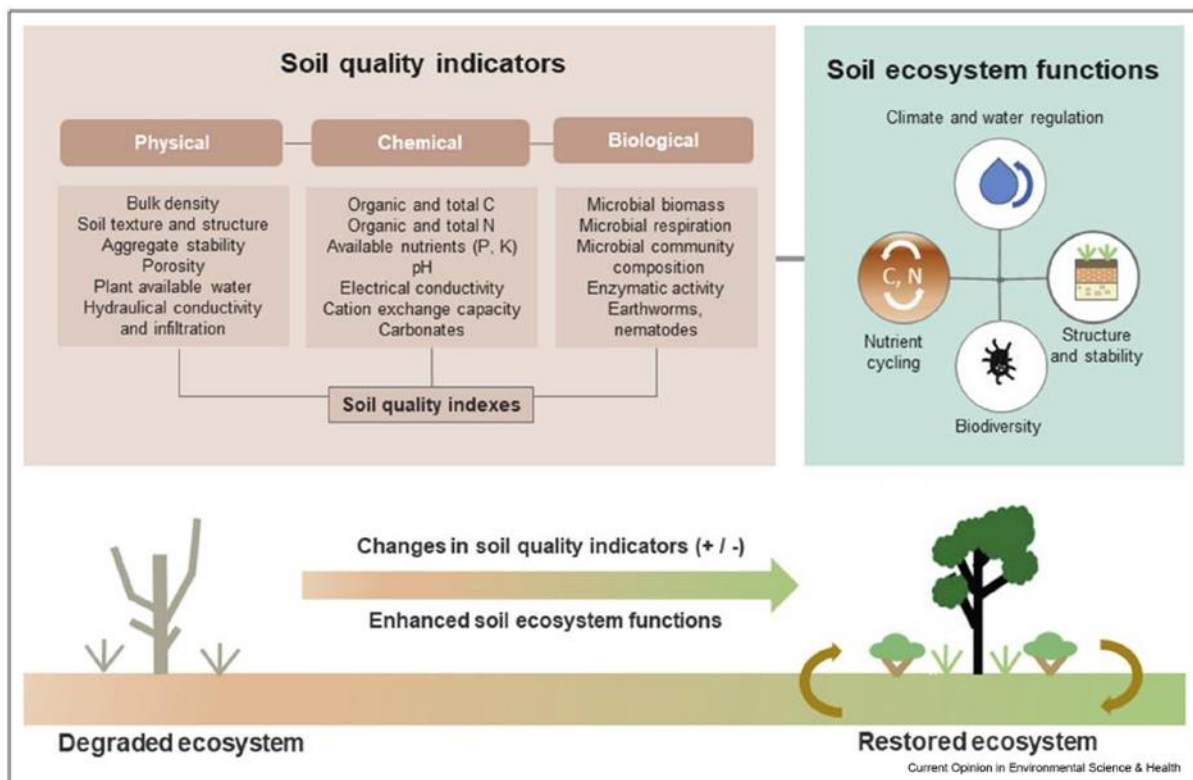


Figure 1.10: Soil quality indicators used to assess soil health (Munoz-Rojas, 2018).

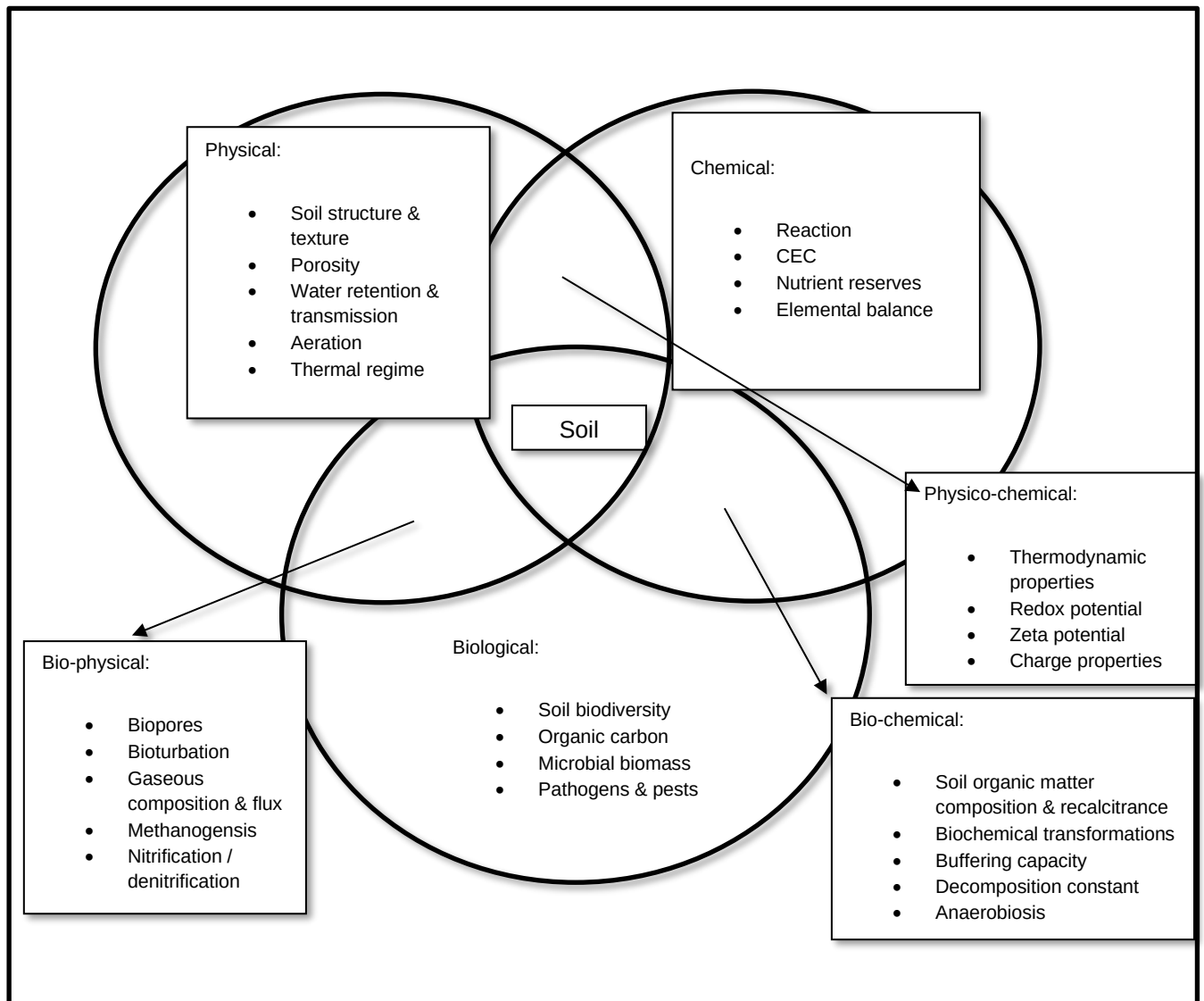


Figure 1.11: Components considered when determining soil health (Bünemann *et al.*, 2018; Cardoso *et al.*, 2013; Ehlers, 2019; Munoz-Rojas, 2018).

#### 1.3.4.2 Bio-indicators and beneficial soil nematodes

Beneficial soil nematodes are quite diverse in their feeding habits (see Table 1.2 and Figure 1.3), activity and function in the soil food web (Buchan *et al.*, 2013; Du Preez *et al.*, 2018; Habig *et al.*, 2018). They feed on a variety of micro-organisms and their basic feeding habits are used to classify them in trophic groups which are fungivores, bacterivores, omnivores and predators.

Beneficial nematodes help with nitrogen fixing, nutrient cycling and can provide insight into soil food web stability (Buchan *et al.*, 2013; Habig *et al.*, 2018; Ugarte *et al.*, 2013). They play a significant role in the decomposition of organic material into nutrients that can be taken up by crops. The connection between beneficial soil nematodes and microbial communities within the same niches and under similar environmental conditions can be used as a bio-indicator of soil

health, as these populations react swiftly to any changes in their environments (Du Preez *et al.*, 2018; Habig *et al.*, 2018; McSorley, 2019).

Soil nematode communities can be used as bio-indicators of the soil condition, which in turn can be used to adapt current activities of an area to promote sustainable soil management (Du Preez *et al.*, 2018; Malherbe & Marais, 2015; Sieriebriennikov *et al.*, 2014). This process of biomonitoring mainly uses nematode taxonomy based on the life cycle/trophic level.

Families, genera and species of herbivorous and beneficial nematodes in soil are divided into five classes referred to as coloniser-persister or the c-p series (Ferris & Bongers, 2009; Sieriebriennikov *et al.*, 2014). A Maturity Index (MI) is designated to each c-p class according to the weighted mean proportion of the beneficial nematode assemblage and indicates the maturity or stability of an ecosystem (Ferris & Bongers, 2009; Neher *et al.*, 2004; Sieriebriennikov *et al.*, 2014). Some important definitions to understand the use of free-living soil nematodes as indicators of soil health are given below:

1. The coloniser-persister (c-p) scale (Table 1.5) is a 1-5 scale assigned to nematodes in soil and freshwater according to their *r* and *K* strategies (Ferris & Bongers, 2009; Ferris *et al.*, 2001).
2. The *r* strategists or 'colonisers' are generally described as relatively small nematodes with short life cycles and probably high reproductive rates – beneficial soil nematodes with c-p values 1 and 2 are grouped as *r* strategists and PPN such as *Tylenchus* Bastian, 1865. There are exceptions where some of these nematodes can develop into large individuals, with longer life cycles and lower reproduction rates (Nemaplex, 2021; Ritz & Trudgill, 1999).
3. The *K* strategists or 'persisters' are usually relatively large nematodes with longer life cycles and lower reproduction rates - beneficial soil nematodes with c-p values 3 to 5 are grouped in this category and PPN such as RKN and Longidoridae (Nemaplex, 2021; Ritz & Trudgill, 1999).

Table 1.5: The Coloniser-persister (c-p) scale for nematodes (Ferris & Bongers, 2009; Ferris *et al.*, 2001).

| c-p value | Description                                                                                                                                                                            | Trophic group                                          |
|-----------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------|
| 1         | Short generation time, small eggs, high fecundity, feed continuously in enriched media, form dauer juveniles as microbial blooms subside                                               | Mainly bacterivores                                    |
| 2         | Longer generation time & lower fecundity than c-p1 group, very tolerant to adverse conditions, may become cryptobiotic, feed more deliberately & continue feeding as resources decline | Mainly bacterivores & fungivores                       |
| 3         | Longer generation time, greater sensitivity to adverse conditions                                                                                                                      | Fungivores, bacterivores & carnivores                  |
| 4         | Longer generation time, lower fecundity, greater sensitivity to disturbance                                                                                                            | Besides other trophic groups, smaller omnivore species |
| 5         | Longest generation, largest body sizes, lowest fecundity, greatest sensitivity to disturbance                                                                                          | Mostly carnivores & omnivores                          |

Sieriebriennikov *et al.* (2014) developed an R-code, representing an advanced and user-friendly online program to help study soil ecosystems, which is named the Nematode Indicator Joint Analysis (NINJA) tool. Indices used to study nematode populations are the c-p and MI as well as the enrichment index (EI), structure indices (SI), channel index (CI), basal index (BI) and various metabolic footprint indices. The MI calculated from the c-p series is used to categorise an ecosystem from class 1 (defined as disturbed/enriched) to class 5 (mature/structured) (Ferris & Bongers, 2009). From this, the health of the soil can be estimated. Terms and definitions used in determining soil health are defined below:

1. Functional guild is defined as a matrix of the feeding habits of nematodes, incorporating their biological, ecological, life history characteristics – all incorporated in the c-p classification (Ferris *et al.*, 2001; Neher *et al.*, 2004).
2. The faunal profile is an indicator of the state of a given food web where the soil is categorised into four quadrats (Figure 1.12) according to the presence, diversity and density of beneficial soil nematodes as affected by stress/disturbances (Ferris *et al.*, 2001; Neher *et al.*, 2004;).
3. Enrichment index (EI) – a measure of opportunistic bacterivore and fungivore nematodes (c-p 1 & 2) present in a given soil substrate (Ferris *et al.*, 2001; Neher *et al.*, 2004).
4. Structure index (SI) – use of beneficial soil nematodes with higher c-p values as an indicator of soil health (Ferris *et al.*, 2001; Neher *et al.*, 2004).
5. Channel index (CI) – indicator of predominant decomposition pathways (fungal/bacterial) that occur in soil food webs where bacterivores and fungivores are used as soil health indicators (Ferris *et al.*, 2001; Neher *et al.*, 2004).
6. Basal index (BI) – indicator of a food web diminished by stress or limited nutrient resources (Ferris *et al.*, 2001).

The metabolic footprint of a nematode (F), an estimator of a nematode's contribution to the different processes in an ecosystem or food chain contains production and respiration components, can be used as an index of the carbon utilisation of component taxa and is a valuable addition to the soil food web analysis (Ferris, 2010; Ferris *et al.*, 2001). The different metabolic footprints are defined as follows:

1. Enrichment footprint – metabolic footprint of nematodes responding most rapidly to resource enrichment (Ferris, 2010).
2. Structure footprint – metabolic footprint of higher trophic levels (may have regulatory function in the food web) which indicate the abundance of organisms (non-nematode taxa) performing similar functions (Ferris, 2010).
3. Functional footprint – the total area of the enrichment and structure footprints on the functional guild analysis graph (Ferris, 2010).
4. Herbivore, Bacterivore and Fungivore footprints – based on the nematode indicators of carbon and energy entering the soil food web through their respective channels (Ferris, 2010).
5. Aggregate footprint – the metabolic footprint of the complete nematode assemblage (regardless of trophic level or ecosystem function) (Ferris, 2010).

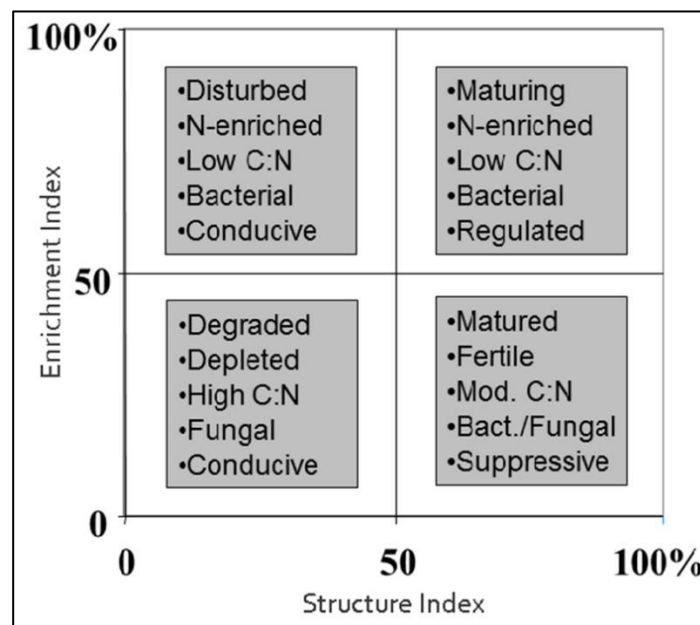


Figure 1.12: A schematic representation of the nematode faunal analysis (Nemaplex, 2021).

#### 1.4 Management strategies

Proper management of nematodes is crucial to ensure that the problem does not escalate and spread into unaffected areas, but most importantly to enable the continued sustainability of not only the potato industry with decent quality produce, but food production as a whole (Habig *et al.*, 2018; Singh *et al.*, 2015). Crop rotation, the use of genetically pest resistant and/or tolerant

cultivars and applying organic soil amendments with nematicidal and fertilisation properties are alternative strategies to control PPN (Jones *et al.*, 2017; Zijlstra *et al.*, 2000).

Due to some of these problems, producers have had to resort to certain phytosanitary practices, such as long fallow periods, to limit the damaging effects of soil-pathogens and maintain the profitability of the potato plantings in those fields (Franke *et al.*, 2011; Van der Waals *et al.*, 2016). The control of PPN may need more intensive methods of control. Generally, chemically derived nematicides are widely used to control nematode infestations, while another option is that potato producers follow good crop rotation practices (Jones *et al.*, 2017). Crop rotation is mostly used for the management of potato scab (Personal communication, Jordaan J., 2017). However, with the increase in nematode problems reported and the rising pressure from governments and environmentalists to reduce or remove certain nematicides from the market, this practice could assist in nematode management (Forghani & Hajihassani, 2020; Ntalli & Caboni, 2012). The use of resistant cultivars could be helpful in this regard, but to date, no commercial breeding for nematode-resistant cultivars has been done in South Africa (Personal communication, Du Raan, C., 2019) and a study into the host-status of some of the popular cultivars planted in South Africa found that these cultivars were excellent RKN hosts (Pofu & Mashela, 2017). Cultivars generally planted in South Africa may be subject to area and season preference (par. 1.2.5).

#### **1.4.1 Chemical control**

Chemical pest and disease control is a multi-billion-dollar global industry that is forecasted to reach US\$ 308.9 billion by 2025 (Fernandez, 2021). Chemical crop protection products are usually highly effective and give the producers faster results compared to cultural and biological control methods (Ntalli & Caboni, 2012). Unfortunately, there are high risks associated with using chemical crop protection products and strict safety procedures and personal protective equipment are needed for safe and efficient application of these products (Forghani & Hajihassani, 2020). The pressure from governments and environmentalists to reduce and ultimately remove certain chemical nematicides from the market has been increasing since the 1970s, to promote the production of pesticide-free produce (safe food) as well as limit the negative effects these nematicides have on the environment (Forghani & Hajihassani, 2020; Jones *et al.*, 2017). In South Africa, 11 active ingredients have been registered as nematicides for use on potato crops (Table 1.6) (Agri-Intel, 2021). Nematicides are divided into fumigants, non-fumigants and biological nematicides (Jones, 2017).

Some producers use these crop protection products in programs (fumigant followed up with a non-fumigant at/post-planting in areas where RKN population densities are high), while others only use either a fumigant pre-plant or a non-fumigant nematicide at and/or post-planting. Very

few innovative solutions have been developed in the last decades, meaning that the nematicides currently available have developed from old and very hazardous active ingredients, which have unfavourable toxicological/eco-toxicological profiles and require high application rates to be effective. This was until the development of fluopyram was completed by Bayer (PTY) Ltd.

Velum® Prime 400 SC was registered in South Africa in 2016 on potato for use against RKN and early blight (*Alternaria solani* Sorauer, 1896) ([https://www.cropscience.bayer.africa/za/en-za/products/product-detail-page.html/insecticides/velum\\_prime\\_400\\_sc.html](https://www.cropscience.bayer.africa/za/en-za/products/product-detail-page.html/insecticides/velum_prime_400_sc.html)) and represents a more environmentally friendly option for local producers. Fluopyram is a unique compound with both broad-spectrum fungicidal and selective nematocidal activity and is the only compound in the new chemical group of pyridinyl-ethyl-benzamides. This active ingredient is the first nematicide with a non-toxic profile for both humans and the environment. Another benefit of using fluopyram is that the application rate is much lower than that of the other chemical nematicides available. The mode of action (MOA) of this compound is through the inhibition of the succinate dehydrogenase (SDH) complex of the mitochondrial respiratory chain of both plant-pathogenic fungi (succinate-coenzyme Q reductase (SQR) and PPN (SDH complex II). This blocks electron transport and energy production in the target organisms. In PPN, the effect of treatment can already be observed after 30 minutes of application, where the affected PPN show more sluggish movement, followed by taking on a hooked or needle-shape as paralysis takes hold. The paralysed PPN remain in this state in the soil until they die (Faske & Hurd, 2015; Oka, 2020). This product was included as a standard nematicide in the trial that formed part of this study.

Table 1.6: Registered nematicides for use on potato in South Africa (Agri-Intel, 2021).

| Active ingredient                  | Trade names                                                                                                                                                                                                                                                         |
|------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| 1,3-dichloropropene                | Destroyer, Dilone AL, Telone II, Tolerance™ 1110 AL, Trimple 1110 AL                                                                                                                                                                                                |
| 1,3-dichloropropene + chloropicrin | Tri-Form 60                                                                                                                                                                                                                                                         |
| Cadusafos                          | Hockey 100 EW, Rugby® 10 G, Rugby® 10 ME, Soccer 100 EW                                                                                                                                                                                                             |
| Chloropicrin                       | Pic Plus, Tripicrin                                                                                                                                                                                                                                                 |
| Ethoprophos                        | AALbuster, Mocap® 150 GR, Phoscap® 15 GR, Procap 15 GR                                                                                                                                                                                                              |
| Fenamiphos                         | Bonfire 400 SC, Farm-Ag Fenamiphos 400 EC, Fenamiphos EC, Fenamiphos 400 EC, NemaCur® 240 CS, NemaCur® 400 EC                                                                                                                                                       |
| Fluopyram                          | Velum® Prime 500 SC, Velum® Prime 400 SC                                                                                                                                                                                                                            |
| Fosthiazate                        | Nemathorin 100 GR                                                                                                                                                                                                                                                   |
| Furfural                           | Crop Guard®, Crop Guard® 80                                                                                                                                                                                                                                         |
| Imidacloprid                       | Sunprid 350 SC                                                                                                                                                                                                                                                      |
| Iprodione                          | Devguard 500 SC                                                                                                                                                                                                                                                     |
| Oxamyl                             | Blockade™ G, Blockade™ SL, Foxamyl 10 GR, Foxamyl 310 SL, Harvest Oxamyl 310 SL, Harvest Oxamyl 100 GR, Oxadate, OxaMyl 210 SL, Oxatak SL, Oxygran 10 GR, Platoon® 100 GR, Platoon® 310 SL, Stetson 310 SL, Strangler SL, Vigour 310 SL, Vydate® 100 GR, Vydate® SL |

## 1.4.2 Alternative strategies for management of plant-parasitic nematodes

### 1.4.2.1 Biological control

Biological control of agro-economically important nematodes refers to the use of biological agents, such as bacteria or fungi (natural parasites/predators) that share the rhizosphere with PPN and are constantly interacting, to reduce the negative effects that nematodes have on the cultivated crops (Strom *et al.*, 2020; Varandas *et al.*, 2020). This type of control has recently enjoyed a lot of attention. Some of the strategies that have been studied and show potential for use include:

1. Predatory nematodes (Dropkin, 1980b).
2. Soil arthropods that feed on nematodes – e.g. mites (Azevedo *et al.*, 2020).
3. Nematophagous fungi and some bacteria species – e.g. *Pochonia chlamydosporia* (Goddard) Zare & W. Gams, 2001, *Trichoderma harzianum* Rifai, 1969, *Purpureocillium lilacinus* (Thom) Samson, 1974, *Pasteuria* Metchnikoff, 1888, *Clostridium* Prazmowski, 1880, *Serratia* Bizio, 1823 (Mazzuchelli *et al.*, 2020; Peiris *et al.*, 2020; Varandas *et al.*, 2020).

### **Bionematicides**

A bionematicide can be defined as a nematicide of biological origin (Glare *et al.*, 2012). Naturally occurring compounds with nematicidal activity can be found in plant extracts (alkaloids, glucosinolates, etc.) and residues or by-products derived from roots, fruits skin, bark, leaves, stems, seeds and flowers (Chelinho *et al.*, 2017; Pino-Otin *et al.*, 2019;). Examples of plant extracts used as bionematicides include Cucurbitacin A and B which can be extracted from e.g. African wild cucumber (*Cucumis africanis* L.) (Dube *et al.*, 2018). Hydrolate extracts from wormwood (*Artemisia absinthium* L. var. Candial) (Pino-Otin *et al.*, 2019) and leaf extracts from goat weed (*Ageratum conyzoides* L.) (Pavaraj *et al.*, 2010) are also used in such formulations.

Research into bionematicides has investigated various bacteria and their metabolites for PPN control, such as various rhizobacteria, *Bacillus* Cohn, 1872, *Pasteuria* and *Pseudomonas* Migula, 1894 (Aballay *et al.*, 2017; Berini *et al.*, 2018, Horak *et al.*, 2019). The use of *Bacillus* spp. for use in agriculture is of particular interest, due to its ability to control PPN, especially RKN (Abbasi *et al.*, 2014; Perez-Garcia *et al.*, 2011; Zhao *et al.*, 2018). Some of the locally available registered bionematicides include Spartacus L7125 (*Beauveria bassiana* (Bals.-Criv.) Vuill., 1912) combined with Romulus L7124 (*T. harzianum* isolate DB104) on carrot (*Daucus carota* L.) and Mytech WP L9077 (*P. lilacinus*) for use on various flowers and vegetables ([www.biologicalcrophealth.co.za](http://www.biologicalcrophealth.co.za)). Studies conducted using *Trichoderma* and *Bacillus* isolates to control PPN on soybean (*Glycine max* (L.) Merr.) and tomato in South Africa showed potential as bionematicides (Chinheya *et al.*, 2017; Engelbrecht *et al.*, 2018). Bayer (Pty) Ltd also produced a bionematicide, namely BioAct,

containing *Purpureocillium lilacinum* (*Paecilomyces lilacinus*) 251, but it is currently not available in South Africa.

#### **1.4.2.2 Soil amendments, cover and green manure crops /organic amendments**

One of the earliest methods suggested to assist in nematode control, was to ensure that the organic contents in the soils were high. This was usually achieved using green manure (working plant residues back into the soil after harvest), improved organic manures and soil amendments obtained from seed oils and oilseed cakes (Dutta *et al.*, 2019; Mojumder, 2000). Soil amendments most likely only improve the soil structure and quality, reducing stress on the plants, although some do exhibit antinematocidal properties (Abdelnabby, 2012; Khalil, 2013). This ultimately leads to healthier crops that naturally resist or outgrow the potential of PPN to attack their root systems while soil amendments do not function as a host, as some cover crops would (Dutta *et al.*, 2019).

Some organic amendments with antinematodal properties include components found in the neem plant, *Azadirachta indica* A. Juss., whether used as green manure, seed oil cake or neem oil extract (Campos *et al.*, 2016; Kosma *et al.*, 2011; Lokanadhan *et al.*, 2012). Little information on the nematocidal effect of neem in terms of nematode control is however available for potato cropping systems. Two studies investigating the use of animal manure and/or neem leaves to control PPN and improve potato yields have shown these strategies to be very promising (Rossi *et al.*, 2021; Shayaa & Hussein, 2019). A study by Hajji and Horrigue-Raouani (2012) also showed improved potato growth and yields, although the treatments did not affect the RKN densities. In potato, the use of manures and neem amendments have yielded inconsistent and variable results (Kimpinski *et al.*, 2003). Successful suppression of nematode populations with a variety of neem amended products and formulations were however observed in various controlled studies on different vegetable crops (Kumar & Khanna, 2006; Singh & Hali, 2017). Field trials mostly show variable and inconclusive results, although two studies have shown that poultry and cattle manure amended with neem extract show significant suppression of RKN populations with an increase in crop production of rice and okra (*Abelmoschus esculentus* (L.) Moench) (Auwal *et al.*, 2015; Collange *et al.*, 2011; Galadima *et al.*, 2015). Neem extract contains various triterpenoids of which azadirachtin is the main compound and is extracted from the plant's seed kernels (Khalil, 2013). Studies have shown that azadirachtin influences insect feeding behaviour and development and is effective against many different insects. Neem products have also been shown to be effective against PPN by reducing the ability of juveniles to hatch from eggs and the mobility of juveniles (Khalil, 2013). When applied as a seed treatment, drench or root dip, tomato plants have shown significantly reduced numbers of RKN J2 and reduced development of galls compared to treatments without neem-based products (Kumar & Khanna, 2006; Singh & Hali, 2017).

The neem-oil amended cow manure granular product, Kalahari 3:1, that was used in the field trial conducted in this study, consists of a combination of 80% cow manure, 10% lime and 10% carbon and is a mixture of Neem oil @ 125 L/ton, Cosmoroot @ 5 g/ton (200N, 87P, 166K g/kg – root stimulant), H85 @ 5 kg/ton (850g/g Humus (carbon)), Soilex @ 5 L/ton (64 g/L organic extracts, 63 g/L carboxylic acids – sodium neutraliser), Mainstay Ca @ 5 L/ton (200 g/L – 42%CaO). Cosmoroot, Mainstay, H85 and Soilex are specialised fertiliser products produced and sold locally by Cosmocel® (<https://cosmocel.co.za>). The mixture and formulation of the Kalahari 3:1 granule product, which is, in essence, an organic fertiliser with a nematicidal component, is facilitated by Organic Fertilizer Manufacturers Botswana (OFMB) (<https://www.organicfmb.com>). This product has thus far been tested in pineapple (*Ananas comosus* (L.) Merr.) in KwaZulu-Natal and tomato in Botswana without conclusive results (Personal communication, Mike Hallam, Managing Director, OFMB, 2018). The composition of this product does have the potential to be a more environmentally friendly option for producers and was therefore investigated in this study for its effect on the existing PPN population at the site near Tzaneen where potato was grown.

Another strategy used to reduce PPN population densities, namely crop rotation with resistant varieties, non-host plants or incorporating fallow periods also aid in the management of other pests and diseases such as potato scab (Personal communication, Jordaan J., 2017; Pederson, 2015). Emmond and Ledingham (1972) stated that the success of crop rotation depends on using non-host plants and a short survival time of the disease-causing agent in the absence of the host crop. Resistant cultivars allow PPN to feed but will inhibit reproduction and in turn, lead to lower PPN population densities. This strategy should not be used continuously and needs to be combined with other control strategies to reduce the risk of selection for other PPN to become dominant (Dutta *et al.*, 2019; Pederson, 2015). This is true in nematode management strategies as well, but as previously stated, PPN encountered in potato cropping systems can infest a wide range of cover and rotational crops which makes effective crop rotation more challenging (Jones *et al.*, 2013; Onkendi *et al.*, 2014).

In South African potato growing areas, producers tend to use different graze crops, mostly grasses, also acting as cover crops between potato plantings (Personal communication, Du Raan, C. (PSA), 2021). Many crop rotation or cover cropping strategies focussed on rotating with crops, e.g. Brassicaceae, that have biofumigant properties (Dutta *et al.*, 2019). However, this was not the focus of this study and is therefore not discussed further. In the study forming part of this project, the field was allowed to recover naturally and the predominant vegetation that grew during the fallow period was White Buffalo grass (*Urochloa mosambicensis* (Hack.) Dandy. (Family: Poaceae)) (Malan, 2018; Mashau, 2010), which is different from what most producers plant in South Africa (Personal communication, Du Raan, C. (PSA), 2021). This is a perennial grass, with

a tufted inflorescence consisting of numerous spike-like racemes arranged alternately on a central axis (Fig. 1.13). It is stoloniferous, which is sometimes rooting and branching from its lower nodes. It is indigenous to Africa, occurring in Kwa-Zulu Natal, spreading northwards up into East Africa. White Buffalo grass is commonly found in disturbed areas, fallow lands and trampled ground. It is considered a palatable grass with average leaf production and is favoured by cattle for grazing. This plant is considered a good indicator of disturbed areas, drought-resistant, preferring sandy or loam soils and summer rainfall (Malan, 2018; Mashau, 2010). A benefit of using White Buffalo grass as a cover crop is that it is reported to have shown a slight degree of galling (<10% of the root system) due to infection by *M. javanica* when grown in combination with *Desmodium ovalifolium* (Guill. & Perr.) in Brazil (Lenné, 1981). No information could however be found regarding the host status of this grass to other *Meloidogyne* spp.



Figure 1.13: White Buffalo grass (*Urochloa mosambicensis* (Hack.) Dandy (<http://pza.sanbi.org/urochloa-mosambicensis>)).

Practices followed by producers can vary greatly depending on their location, resources, disease management needs, whether they are planting table or seed potato and more (Steyn *et al.*, 2016; Van der Waals *et al.*, 2016). A standard 4-year rotation is followed by most producers in South Africa, where the potato crop will be rotated with maize or soybean (*Glycine max* L.) and then planted with a grass crop for a year (resting year for the soil) before the next potato planting. This strategy is implemented frequently in the Mpumalanga province. Variations do occur depending on the needs of the producer (Personal communication, Riley, R., Agronomist, 2020). In the irrigation areas of the North-West and Free State provinces, producers follow a similar rotation, but would use rotation crops such as sunflower (*Helianthus annum* L.), onion (*Allium cepa* L.) and sometimes alfalfa (*Medicago sativa* L.) (Personal communication, Wethmar, J., Agronomist,

2020). Producers in the Sandveld area rotate with cereal crops or onion and most producers follow longer rotation cycles, with one producer planting potato for 4 years followed by onion and cereals (oat: *Avena sativa* L.) for the next 4 years before planting potato again (Personal communication, Baloyi, A., Agronomist, 2020). These crop rotation cycles are generally not only implemented for PPN management, but also to reduce pressure from other diseases such as potato common scab (*Streptomyces scabies*, Lambert & Loria) and to rest the soil between plantings (N'Dayegamiye *et al.*, 2017; Wright *et al.*, 2016). In the Limpopo province, the crop rotation cycles differ greatly between the different climatic zones. In the Dendron-Polokwane area, producers follow a 3-5-year rotation. Most producers plant cover crops used for cattle grazing until the next potato planting, while others include an onion planting after the potato crop and a fallow year before planting potato again. Producers located in the warmer Bushveld of Limpopo have different strategies, although table/process potato farmers follow a 5-year rotation and seed potato producers follow a 7-year rotation. Large scale producers tend to plant onion and feed or fallow until the next planting, while smaller producers rotate potato with onion or a bean (Fabaceae) variety and then plant a sequence of vegetable crops before leaving the soil naturally fallow before the next planting. This is done to supplement cash flow in most cases (Personal communications: Van Zyl, A., Area Manager, 2020 & Jordaan, J., Producer, 2017).

#### **1.4.2.3 Other strategies**

Other non-chemical strategies that can be implemented by local potato producers include soil solarization and trap crops.

- Soil solarization:
  - Clear polyethylene sheets are placed over moist soil (summer) to increase topsoil temperature (>50 °C) (Dutta *et al.*, 2019).
  - This practice is not practical or cost-effective for large-scale/commercial producers. In addition, it is expensive and weather conditions could counteract sterilisation effects, while pathogenic and beneficial microbes will be killed (Dutta *et al.*, 2019).
- Trap crops:
  - Suitable for small-scale crop production
  - A trap crop is destroyed after J2 infection, before females can lay eggs (Sasanelli *et al.*, 2021).
- Sanitation practices:
  - Prevent and reduce the spread of PPN to other fields.
  - Clean equipment and shoes are prerequisites for this practice to be effective, while soil must be removed from vehicles and implements before moving from one field to the next.

- Plant certified clean planting material (Dufour *et al.*, 2003; Sasanelli *et al.*, 2021).
- Plastic mulching:
  - Red plastic mulch promotes growth above ground, limiting food production for PPN in the soil.
  - Similar effect to that of soil solarization expected.
  - Can be expensive and labour intensive to install, especially on a large scale (Dufour *et al.*, 2003).

It is essential to properly integrate the existing and new knowledge obtained from research (from identification of nematodes to new agricultural tools and strategies) in the application of management strategies. These strategies ensure the sustainable and effective reduction of PPN population densities. This was attempted in the study, firstly by accurately identifying the RKN species dominant in the field (Chapter 2) and secondly by evaluating the effect of Kalahari 3:1 against PPN populations (Chapter 3) as well as the effect of this product and grass fallow on the beneficial soil nematodes present in the field (Chapter 4).

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## Chapter 2: Morphological and molecular identification of *Meloidogyne* spp. from a potato (*Solanum tuberosum* L.) field

### 2.1 Introduction

Effective control strategies, including both chemical and alternative methods, for the management of plant-parasitic nematode (PPN) populations in any agricultural cropping system rely heavily on the accurate identification of the PPN species present (Dos Santos *et al.*, 2019; Varandas *et al.*, 2020).

There are different ways to identify PPN and determine the various species that may occur in a community (containing more than one species; Rashidifard *et al.*, 2019b), viz morphological, biochemical and molecular identification. Morphological identification of PPN of the same genus to species level is considered a highly specialised field of expertise (Marais *et al.*, 2017; Qing *et al.*, 2020). Nematodes are morphologically conservative and certain characteristics will differ little in and between populations (containing one species; Rashidifard *et al.*, 2019b) and/or communities. Some of the characteristics of various life stages of the species needed to be studied for accurate identification include, but are not limited to, body length, size and shape of the stylet, lateral lines, size and shape of the head and tail, type of reproductive system, the position of the vulva and type of habitus (body shape of a dead individual). In the case of root-knot nematodes (RKN; *Meloidogyne* Goeldi, 1982), the perineal pattern of mature females is also studied and represents a valuable characteristic (Da Cunha *et al.*, 2018; Karssen *et al.*, 2013; Kleynhans *et al.*, 1996) but is a challenging task due to a high level of expertise and vast experience that is needed (Da Cunha *et al.*, 2018; Oliveira *et al.*, 2011). Even then, inaccurate species identification can be made when using only this approach (Carneiro *et al.*, 2016; Collett *et al.*, 2021).

Molecular techniques used for nematode species identification, either solitary or in conjunction with morphological taxonomy, is an established and developing field (Da Cunha *et al.*, 2018; Dos Santos *et al.*, 2019). The use of DNA-based technologies has become an integral part of identifying RKN species (Hu *et al.*, 2011). These techniques mainly include PCR amplification of mitochondrial DNA (mtDNA, COI), ribosomal DNA (rDNA) intergenic regions (IGS) (Seesao *et al.*, 2017; Tigano *et al.*, 2010) and others (Collett *et al.*, 2021; Rashidifard *et al.*, 2018), followed by restriction fragment length polymorphisms (RFLP) and sequencing. Also species-specific approaches like sequence-characterised amplified region PCR (SCAR-PCR) (Rashidifard *et al.*, 2018; Zijlstra *et al.*, 2000) and real-time PCR (Berry *et al.*, 2008) based on specific probes can be used.

The aim of this study was to use a combined approach of morphology and molecular techniques to identify the predominant RKN species found in the experimental field site in the Tzaneen area where the potato (*Solanum tuberosum* L.) experiment was conducted for this study. This field had not been used for any agricultural practices for fifteen years before this study was initiated.

## **2.2 Materials and methods**

### **2.2.1 Collection and rearing of *Meloidogyne* spp.**

For species identification, RKN present in samples from the trial field were reared *in vivo* in 10 litre plastic pots planted in roots of tomato (*Solanum lycopersicum* L.) seedlings cv. Rodade (a highly susceptible tomato cultivar according to Fourie *et al.* (2012)). After five months, when sufficient egg masses and females were observed on exposed roots removed from the rearing pots, enough root pieces were collected to enable the isolation of sufficient numbers of mature RKN females for both molecular and morphological identification techniques used, as explained in the discussion below.

### **2.2.2 Isolation of mature *Meloidogyne* spp. females**

Infected roots containing egg-laying females collected from the *in vivo* rearing plastic containers were rinsed with clean tap water to get rid of soil particles and debris. Mature females were removed from root galls with tweezers and a scalpel and 20 females were placed into a single 1.5 ml Eppendorf tube containing 20 µl distilled water for DNA extraction. Two such tubes were prepared; hence, 40 females were used for identification purposes. Samples were stored at -10 °C until analyses could be conducted.

For morphological studies, the rest of the infected roots collected were rinsed and then stained using an adapted lacto-glycerol acid-fuchsin staining method (Byrd *et al.*, 1983; Van Bezooijen, 2006) which is described below.

The lacto-glycerol acid-fuchsin staining method (see Figure 2.1) was conducted as follows:

#### **1. Preparation of lacto-glycerol and lacto-glycerol acid-fuchsin solutions:**

- Lacto-glycerol solution (1 L) for de-staining: 333.3 ml lactic acid, 333.3 ml glycerol and 333.3 ml water were added together in a glass flask and mixed until the solution was homogenous.
- Lacto-glycerol acid-fuchsin solution (1 L) for staining: 333.3 ml lactic acid, 333.3 ml glycerol, 333.3 ml water and 0.5 g acid-fuchsin powder were added together in a glass flask and mixed until the solution was homogenous and purple in colour.

#### **2. Staining of galled roots:**

- Lacto-glycerol acid-fuchsin solution (400 ml) was heated in a glass beaker, in a fume hood on a heating pad until boiling.
- Galled roots were submerged in the boiling solution for 2.5 minutes. Larger, more heavily galled roots were submerged for 3 minutes.

### 3. De-staining of galled roots:

- Stained galled roots were placed in a plastic 100-ml capacity container and covered in lacto-glycerol solution for 24 hours.
- After 24 hours, the roots were inspected under a stereomicroscope and stained female nematode specimens were removed with a scalpel and needle tip and isolated for cutting of the perineal pattern area (described in paragraph 2.2.3.1).

### 4. Storage and preservation of stained galled roots:

- Roots were placed back into the container with lacto-glycerol and stored in a cool dry environment.

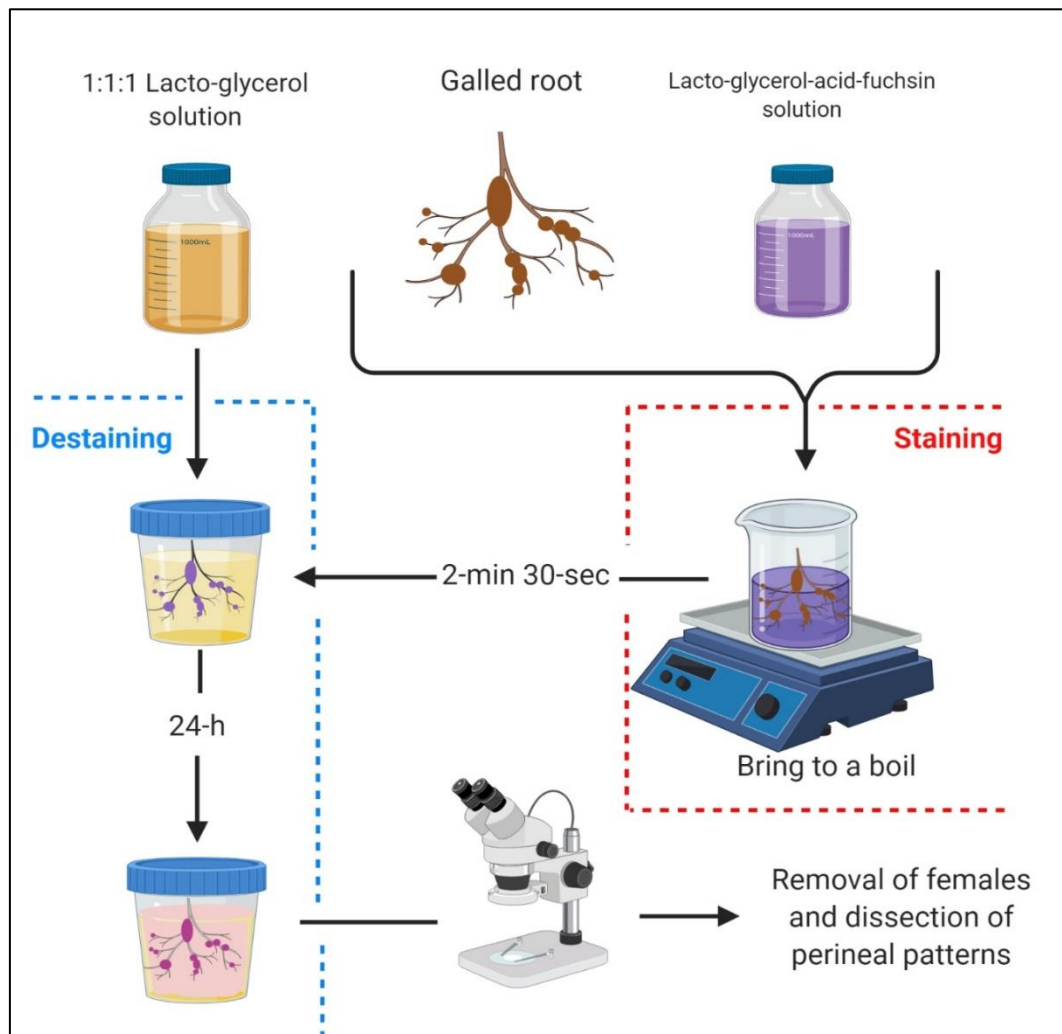


Figure 2.1: Diagram depicting the lacto-glycerol acid-fuchsin staining method used to stain root-knot nematode individuals inside tomato roots (Courtesy of Raymond Collett, NWU).

### **2.2.3 Morphological identification of *Meloidogyne* spp. females**

#### **2.2.3.1 Oesophageal and perineal-pattern characteristics used for *Meloidogyne* spp. identification**

Using a dissecting microscope, mature females were removed from the roots, using a 14 cm, stainless-steel dissecting (teasing) needle with a plastic handle. The protocol followed for this activity was described by Marais *et al.* (2017). The females were moved to a small drop of lacto-glycerol in a petri dish lid. The posterior part of the nematode was removed with a scalpel using a paper-cutting action. The body tissue was gently removed from the posterior part and the cuticle was subsequently cut into a square with the perineal pattern in the centre. The perineal pattern consists of the anus and vulva (perineum), lateral lines, cuticular striae, tail terminus and phasmids (Hirschmann, 1985; Karssen & Van Aelst, 2001).

The perineal pattern and corresponding head region of each female specimen was then placed onto a microscope slide in a drop of glycerine (2 females per slide), covered with a cover slip and sealed with clear Cutex nail polish (Marais *et al.*, 2017). This was repeated with 40 females to ensure that at least 21 identifications could be made. Microscope slides were subsequently investigated using a light microscope (100x magnification) and each specimen was identified to species level. This was executed at the NWU Ecorehab laboratory with the assistance of Prof Driekie Fourie and MSc student at the time, Raymond Collett. The identity of each female was recorded by using characteristics from protocols described by Brito *et al.* (2004), Karssen *et al.* (2013), Kleynhans (1991), Rashidifard *et al.* (2019a) and Yang and Eisenback (1983) (Table 2.1).

### **2.2.4 Molecular identification of mature *Meloidogyne* spp. females**

#### **2.2.4.1 DNA extraction and SCAR-PCR**

Deoxyribonuclease (DNA) was extracted from the 20 females placed in each of the Eppendorf tubes (Section 2.2.2) using a protocol described by Musapa *et al.* (2013), known as the chelex-100 protocol. This was achieved by adding 3 µl proteinase K (20 mg<sup>-1</sup>) and 20 µl chelex (5% w/v) to each Eppendorf tube that contained the 20 mature RKN females, after which the contents were agitated using a vortex for 1 minute and centrifuged for 30 seconds (@ 13 500 mv) to allow the sediment to settle in the bottom of the tubes. These tubes were incubated at 56 °C for 2 hours, followed by 10 minutes at 95 °C after which the tubes were stored at -20 °C until they were used.

Table 2.1. Description of morphological characteristics used for *Meloidogyne* spp. females.

| <i>Meloidogyne</i> spp.                                     | Perineal pattern morphology <sup>1,2,3,4,5,</sup>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                | Shape of the lumen of the esophagus <sup>1,3</sup>                                                                                                         |
|-------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <i>M. enterolobii</i> , Yang and Eisenback, 1983            | Round to ovoid; dorsal arch moderately high to high; apex usually rounded but square-like in some specimens; distinct phasmids in the tail-terminus, with striae found around phasmids in South African populations; lateral lines are usually lacking and/or not distinct with the exception that distinct lateral lines (similar to <i>M. javanica</i> ) were observed occasionally in <i>M. enterolobii</i> specimens of a Florida and Mexico population, respectively; perivulval area is generally free of striae; striae on the ventral area of pattern generally finer and smoother; tail tip is visible. | No information was recorded for the lumen of oesophagus form/shape in pro- and metacarpus to be used as a discriminatory characteristic.                   |
| <i>M. incognita</i> (Kofoed and White, 1919) Chitwood, 1949 | Circular to oval; dorsal arch medium-high to high; apex broadly rounded or squarish; tail terminus clear/some disordered phasmids.                                                                                                                                                                                                                                                                                                                                                                                                                                                                               | Lumen lining expands, then narrows towards spheroid; occasionally visible as an ovoid metacarpus lining.                                                   |
| <i>M. javanica</i> (Treub, 1885) Chitwood, 1949             | Circular to oval; dorsal arch low to medium-high, apex squarish to broadly round; areas above lateral lines not bulged outwards; lateral lines usually visible as distinct double lines; distinct rectal punctuations also visible.                                                                                                                                                                                                                                                                                                                                                                              | Procorpus lining is usually cylindrical but may expand/narrow immediately in front of the metacarpus lining which is usually ovoid, occasionally spheroid. |

<sup>1</sup>Kleynhans (1991); <sup>2</sup>Yang & Eisenback (1983); <sup>3</sup>Brito *et al.* (2004); <sup>4</sup>Karssen *et al.* (2013); <sup>5</sup>Rashidifard *et al.* (2019a).

The extracted DNA was then amplified with the Vacutec thermocycler ([www.vacutec.co.za](http://www.vacutec.co.za)). The following process was followed:

1. Prepare 25 µl PCR mix in clean Eppendorf tubes:
  - The PCR mix was prepared by adding 12.5 µl ready-to-use Master mix (Promega Corporation, USA) + 1 µl forward primer (10 µM) (for each of the respective *Meloidogyne* spp.) + 1 µl reverse primer (10 µM) (for each of the respective *Meloidogyne* spp.) + 5 µl extracted DNA + 5.5 µl ddH<sub>2</sub>O together.
2. The following program was adhered to during the PCR process for each of the forward and reverse primers used for SCAR-PCR in the thermocycler:
  - Initial denaturation: 2 minutes at 94 °C followed by 35 cycles of 30 seconds at 94 °C.
  - Annealed for 30 seconds at the respective temperatures for each primer, followed by 1 minute at 72 °C (Rashidifard *et al.*, 2019b; Long *et al.*, 2006; Zijlstra *et al.*, 2000):
    - Ff/Rf primers = 58 °C
    - Fh/Rh and Fc/Rc primers = 60 °C
    - Far/Rar primers = 61 °C
    - Finc/Rinc primers = 54 °C
    - Fjav/Rjav and Fme/Rme primers = 64 °C

The primer sequences listed in Table 2.2. and used by Visagie *et al.* (2018) to identify South African populations of *Meloidogyne javanica* (Treub, 1885; Chitwood, 1949), *Meloidogyne incognita* (Kofoid & White, 1919; Chitwood, 1949); *Meloidogyne arenaria* (Neal, 1889; Cobb, 1890); and *Meloidogyne enterolobii* (Yang & Eisenback, 1983), were also used in this study. Also the DNA of these RKN species identified by Visagie *et al.* (2018) were used as the reference standards in this study to determine which species were present in the trial site (Rashidifard *et al.*, 2019b; Visagie *et al.*, 2018).

Table 2.2: Primer code, sequence, specificity, amplification size and source of primers used for the molecular identification of *Meloidogyne* populations from the trial site.

| Primer code      | Primer sequence                | Primer specificity    | Fragment size | Reference                     |
|------------------|--------------------------------|-----------------------|---------------|-------------------------------|
| <sup>1</sup> Far | TCG GCG ATA GAG GTA AAT GAC    | <i>M. arenaria</i>    | 420           | Zijlstra <i>et al.</i> (2000) |
| <sup>2</sup> Rar | TCG GCG ATA GAC ACT ACA ACT    |                       |               |                               |
| FMe              | AAC TTT TGT GAA AGT GCC GCT G  | <i>M. enterolobii</i> | 250           | Long <i>et al.</i> (2006)     |
| RMe              | TCA GTT CAG GCA GGA TCA ACC    |                       |               |                               |
| Finc             | CTC TGC CCA ATG AGC TGT CC     | <i>M. incognita</i>   | 1200          | Zijlstra <i>et al.</i> (2000) |
| Rinc             | CTC TGC CCT CAC ATT AGG        |                       |               |                               |
| Fjav             | GGT GCG CGA TTG AAC TGA GC     | <i>M. javanica</i>    | 700           | Zijlstra <i>et al.</i> (2000) |
| Rjav             | CAG GCC CTT CAG TGG AAC TAT AC |                       |               |                               |

<sup>1</sup>F=Forward primer; <sup>2</sup>R=Reverse primer

#### 2.2.4.2 Gel electrophoresis

Once DNA amplification was completed, a gel electrophoresis process was set up to determine the quality of the extracted RKN female's DNA. This was accomplished by preparing a 1% agarose gel with TAE buffer and loading an O'GeneRuler™ 1kb DNA ladder in the first well of the gel followed by loading 4 µl of PCR products obtained (Section 2.2.4.1) for the RKN females investigated to the subsequent wells of the gel and electrophoresing the gel for 50 minutes at 80 V. The ladder was used to indicate the size of the DNA bands (amplification fragment size) of each RKN species found in the trial site. To observe the DNA bands of the RKN females used in the gel, the DNA was stained with GelRed ([www.biotium.com](http://www.biotium.com)) and placed under a UV transilluminator.

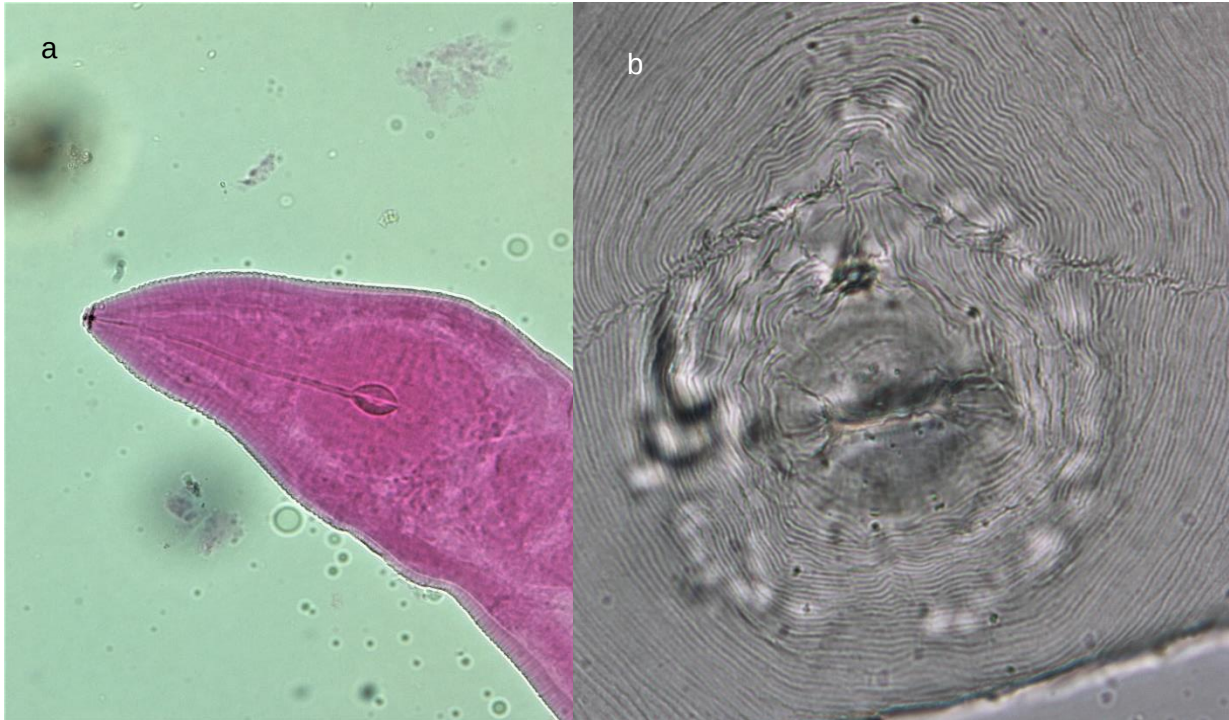
### 2.3 Results

#### 2.3.1 Morphological identification of *Meloidogyne* spp. females

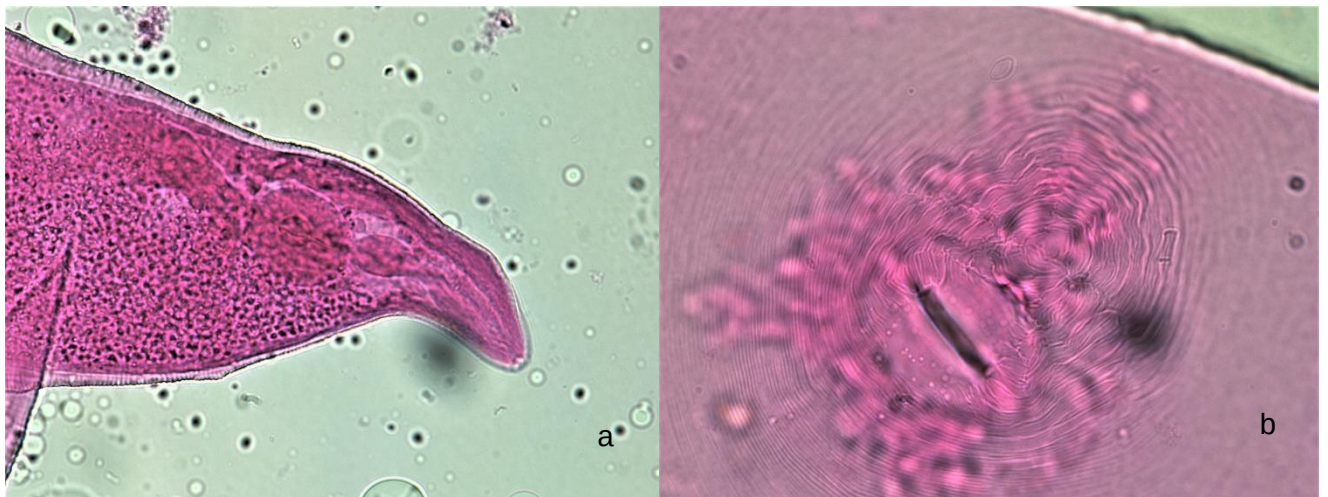
From the perineal patterns and oesophageal characteristics observed for the RKN females isolated from the tomato roots, two species were prevalent in the study site, namely *M. javanica* (representing 90% of the samples) and *M. enterolobii* (representing 10% of the samples). Identification was done by using these two characteristics described in the protocols of Brito *et al.*

(2004), Karssen *et al.* (2013), Kleynhans (1991), Rashidifard *et al.* (2019a) and Yang and Eisenback (1983), as listed in Table 2.1.

Oesophageal characteristics with corresponding perineal patterns of *M. javanica* are shown in Figure 2.2. The perineal patterns with corresponding oesophageal characteristics of *M. enterolobii* females are shown in Figure 2.3.



Figures 2.2a & b: Oesophageal characteristics (a) and a perineal pattern (b) of *Meloidogyne javanica* females isolated from tomato roots grown in infested soil from a field where a potato experiment was conducted near Tzaneen, Limpopo province, South Africa. (Photo's courtesy of Raymond Collett).



Figures 2.3a & b: Oesophageal characteristics (a) and a perineal pattern (b) of *Meloidogyne enterolobii* females isolated from tomato roots grown in infested soil from a field where a potato experiment was conducted near Tzaneen, Limpopo province, South Africa. (Photo's courtesy of Raymond Collett).

### 2.3.2 Molecular identification of *Meloidogyne* spp. females

The morphological identification of the *Meloidogyne* spp. community present at the Tzaneen trial site where this research was conducted was subsequently supported by the results of the SCAR-PCR which showed that *M. enterolobii* (Figure 2.4a) and *M. javanica* (Figure 2.4b) were the species present in the soil and root samples collected.

The gel electrophoresis visualisation of the PCR products clearly showed that the samples amplified with the *M. enterolobii*-specific SCAR primer amplified the 250 bp *M. enterolobii* SCAR fragment which was analogous to that of the *M. enterolobii* standard. During the PCR reaction of samples annealed with the *M. javanica*-specific primer, the species-specific fragment amplified was 700 bp in size which corresponded with the *M. javanica* standard.

None of the PCR reactions containing *M. incognita* or *M. arenaria*-specific SCAR primers respectively, yielded 1200 bp or 420 bp SCAR fragments, indicating that these species were not present in the samples collected from the trial field.

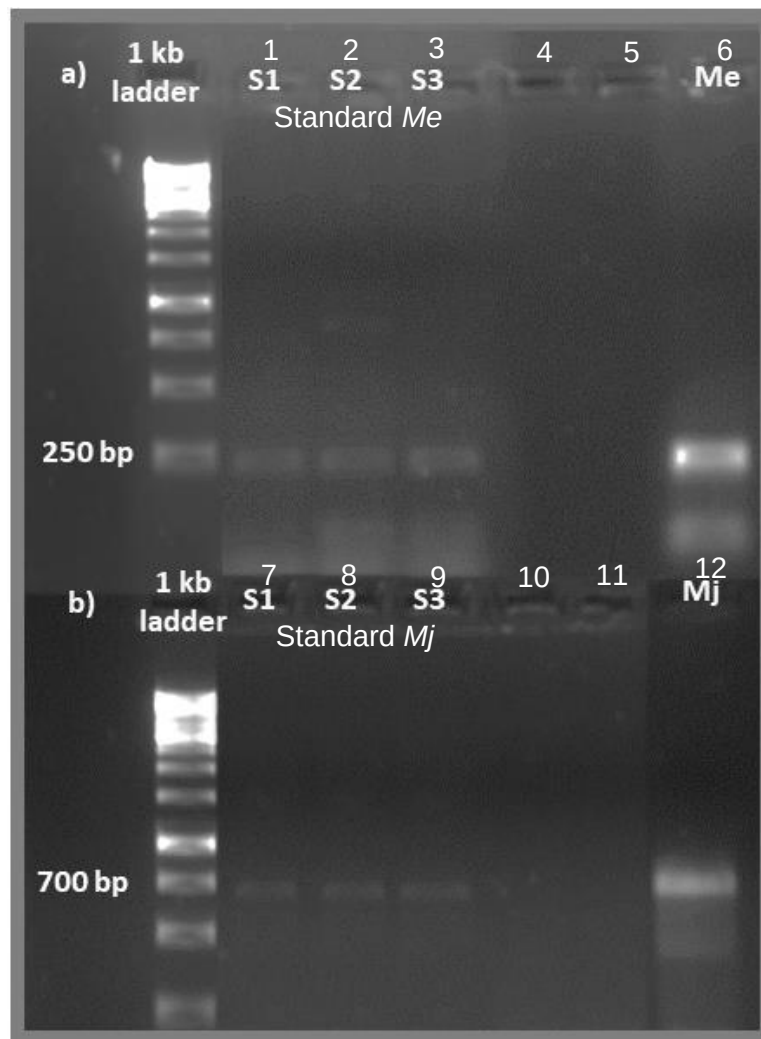


Figure 2.4: SCAR-PCR gel electrophoresis results showing positive results for a) *M. enterolobii* (Me) and b) *M. javanica* (Mj). (Photo courtesy of Gerhard Engelbrecht, NWU). Wells 1-3 represent the standard used with the DNA band for *M. enterolobii* at 250 bp and well 6 represents the *M. enterolobii* DNA bands and line. Wells 7-9 represent the standard used with DNA band for *M. javanica* at 700 bp and well 12 represent the *M. javanica* bands and line.

## 2.4 Discussion

The two RKN species identified from the study field, namely *M. javanica* (which was predominant with a 90% occurrence) and *M. enterolobii* (present in 10% of the samples), had previously been reported from the Limpopo province where this study area is located on a range of crops (Marais & Swart, 2002 & 2003; Rashidifard *et al.*, 2019b). *Meloidogyne javanica* has been identified from below-ground parts of agri- and horticultural crops, grasses, ornamentals and weeds in Limpopo (Marais & Swart., 2002 & 2003; Onkendi & Moleleki, 2013; Rashidifard *et al.*, 2018 & 2019b; Visagie *et al.*, 2018). On the other hand, the occurrence of *M. enterolobii* was limited to roots of guava (*Psidium guajava* L.) and tomato crops in this province (Rashidifard *et al.*, 2018 & 2019b; Visagie *et al.*, 2018). This supports findings from other studies that indicated that *M. javanica*

tends to occur frequently on potato and other crops across South Africa, while the presence of *M. enterolobii* indicates that this species could also be present in more crop producing areas in this province (Onkendi *et al.*, 2014; Rashidifard *et al.*, 2019b; Visagie *et al.*, 2018). This report for *M. enterolobii* from the Limpopo province for the Tzaneen study site is therefore a new addition for potato to the South African Plant Parasitic Nematode Survey database (SAPPNS).

*Meloidogyne javanica* is one of the dominant RKN species found in potato growing regions of South Africa as reported by De Waele and Elsen (2007) and Jones *et al.* (2017). Marais *et al.* (2015) found it to be prevailing in 13 of the 16 potato growing regions in South Africa, namely Ceres, South-Western Cape, Sandveld, Eastern and North-Eastern Cape, Northern Cape, Western Free State, Eastern Free State, Kwa-Zulu Natal, North-West, Gauteng, Loskop Valley and Mpumalanga. The results from this study differ from those of Engelbrecht (2012), where *M. incognita* was found to be the dominant RKN species in the potato fields sampled in the Eastern Free State, while from this study's trial site, no *M. incognita* individuals were recorded.

The other RKN species present in the potato field during this study was *M. enterolobii*, which is regarded as one of the most aggressive emerging RKN pests worldwide (Castagnone-Sereno, 2012; Jones *et al.*, 2013). This species was first reported in South Africa by Willers in 1997, in Mpumalanga, on guava (*Psidium guajava*) and has been found in many other countries worldwide (Collet *et al.*, 2021). Even though this species has been studied extensively since its discovery, both morphologically and molecularly, successful management remains difficult, mainly due to its ability to render resistance genes (that are effective against its thermophilic counterpart species, e.g. *M. arenaria*, *M. incognita*, *M. javanica*) ineffective (Castagnone-Sereno, 2012). In 2013, Onkendi and Moleleki (2013) extracted RKN from 78 different infected potato tuber samples from the different growing regions in South Africa and found *M. enterolobii* only from samples collected in the KwaZulu-Natal province. They concluded that the high rate of reproduction and ability to render resistance genes against other thermophilic species ineffective, makes *M. enterolobii* a threat to potato production both locally and globally (Brito *et al.*, 2004; Onkendi & Moleleki, 2013).

Other RKN species associated with potato in South Africa include *M. acronea* Coetzee, 1956, *M. chitwoodi* Golde, O'Bannon, Santo & Finley, 1980, *M. arenaria*, *M. hapla* Chitwood, 1949 and *M. incognita* (Jones *et al.*, 2017; Marais *et al.*, 2015; Onkendi *et al.*, 2014). These species were not found at the Limpopo site where this research was conducted. In potato growing regions in other parts of the world, *M. incognita* is considered the most damaging and dominant RKN species. In the temperate areas, *M. chitwoodi*, *M. hapla*, *M. minor* (Karssen *et al.*, 2013) and *Meloidogyne fallax* (Karssen, 1996) have been reported, while *M. incognita*, *M. javanica*, *M. arenaria* and *M.*

*enterolobii* are considered as the most important species in tropical and subtropical potato growing regions (Niere & Karuri, 2018).

Studying the oesophageal parts and perineal patterns of the RKN extracted resulted in relatively easy identification of mature *M. javanica* females, but due to the similarity between *M. enterolobii* and *M. incognita* female perineal patterns (Brito *et al.*, 2004; Conceição *et al.* 2012), it was more challenging to accurately identify these species using morphological characteristics. However, Kleynhans (1991) described that, for *M. enterolobii*, the female stylet knobs are rounded and sloping backward slightly with each knob having distinct longitudinal grooves; thus, each knob looks like two in contrast to the stylet knobs of *M. incognita*. The oesophageal photos (Figures 2.2 a. and 2.3 a.) included for the morphological identification of these nematode species did not clearly show these characteristics. It is difficult to capture such characteristics due to the dark staining and the challenges experienced to get all the structures in focus in one photo. Most of the other morphometric characteristics used to distinguish *M. enterolobii* from other species overlap, indicating that the classical morphological identification methods alone are not sufficient to accurately differentiate between species (Kiewnick *et al.*, 2009; Rashidifard *et al.*, 2019a). However, Brito *et al.* (2004) and Rashidifard *et al.* (2019a) recorded that the phasmids in the tail terminus on the perineal pattern are very distinct/pronounced measuring 2-3µm in diameter, which is larger than that of *M. incognita*, *M. javanica* and *M. hapla*. These specific characteristics were therefore valuable tools that were used in this study to discriminate the oesophageal areas and perineal patterns of *M. enterolobii* from those of *M. javanica* from the Tzaneen site where this project was conducted.

Ultimately, the molecular results verified the morphological findings of this study, namely that only *M. javanica* and *M. enterolobii* were present in this potato field as a mixed community. Accurate results obtained in this study for the identification of the latter species hence relied on integrating the use of both morphological characterisation and molecular techniques. While molecular techniques have the advantage of presenting faster results, with the ability of being automated, the results need to be corroborated with the morphological characteristics (Hallman & Meressa, 2018; Rashidifard *et al.*, 2019b; Visagie *et al.*, 2018).

## **2.5 Conclusion**

Utilising both morphological and molecular techniques to accurately identify RKN is critical to address the inconsistencies regarding RKN species identification that could be found in the results of each method on its own. The results for this study showed conclusively that *M. javanica* dominated, followed by *M. enterolobii* were the RKN species present in this potato field. These results will aid in planning the more effective implementation of control strategies to reduce RKN

population densities and increase the sustainability of potato production, not only in the Limpopo province but also across other potato production areas in South Africa.

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## Chapter 3: Effects of a neem-amended product on plant-parasitic nematode population densities present in a potato field

### 3.1 Introduction

Proper management of plant-parasitic nematodes (PPN) is crucial for high quality and sustainable food production (Ibrahim *et al.* 2019; Nicol *et al.*, 2011). Farmers who produce crops susceptible to the PPN species present in the field, such as potato (*Solanum tuberosum* L.), in crop rotation systems, rely heavily on chemical pest and disease control options to improve crop quality and yields (Jones, 2017; Peiris *et al.*, 2020).

Worldwide, the predominant PPN genera that cause potato quality and yield losses include root-knot nematodes (RKN: *Meloidogyne* Goeldi, 1982), cyst nematodes (GCN: *Globodera* (Skarbilovich, 1959; Behrens, 1975) and lesion nematodes (LN: *Pratylenchus* Filipjev, 1936) (Niere & Karuri, 2018). In South Africa, RKN are referred to as the most important nematode pest of potato with *Meloidogyne incognita* (Kofoed & White, 1919; Chitwood, 1949) and *Meloidogyne javanica* (Treub, 1885; Chitwood, 1949) recently considered as the most damaging species (Jones *et al.*, 2017). *Meloidogyne enterolobii* has also been identified from potato in South Africa, but only in the KwaZulu Natal province before this study (Onkendi & Moleleki, 2013), while other species known to infect potato in South Africa include *M. acronea* (Coetzee, 1956), *M. arenaria* (Neal, 1889; Cobb, 1980), *M. chitwoodi* (Golden *et al.*, O'Bannon, Santo & Finley, 1980) and *M. hapla* (Chitwood, 1949) (Marais *et al.*, 2015).

To ensure that the most effective control methods are applied for effective PPN management, it is important to know which species are present and what the population density of each species is (Marais *et al.*, 2017). The most appropriate control strategy to manage RKN in potato fields can be selected from a variety of options, amongst others chemical control which is most widely used, biological agents, host plant resistance or cultural and/or chemical control (Jones *et al.*, 2017; Niere & Karuri, 2018). Although a definite shift towards more environmentally friendly strategies is globally experienced to control RKN in agricultural fields (Fourie & De Waele, 2019), including potato, chemical control is still the most popular strategy applied to reduce densities of these pests in the short-term (Jones, 2017; Niere & Karuri, 2018; Peiris *et al.*, 2020).

Globally, there are many chemically synthesised nematicides (fumigant and non-fumigant) available to combat or reduce the damaging effect that nematodes have on crops. In South Africa, there are 10 active ingredients registered for use against nematodes in potato (Chapter 1, Table

1.6). Nematicides sold in South Africa for use in potato between 2016-2019 are listed in Table 3.1, indicating the estimated volumes for each trade name.

Table 3.1: Estimated volumes (%) of nematicides sold in South Africa, in potato from 2016-2019.

| Product Trade Named                  | Active ingredient                      | Percentage of total estimated volume sold |                  |                  |                  |
|--------------------------------------|----------------------------------------|-------------------------------------------|------------------|------------------|------------------|
|                                      |                                        | 2016                                      | 2017             | 2018             | 2019             |
| <b>VYDATE® 310SL</b>                 | Oxamyl (carbamate)                     | 9.11                                      | 6.07             | 5.04             | 5.12             |
| <b>VYDATE® 100GR</b>                 | Oxamyl (carbamate)                     | 0.45                                      | 0.42             | 0.31             | 0.31             |
| <b>VIGOUR 310SL</b>                  | Oxamyl (carbamate)                     | 0.10                                      | 1.68             | 1.43             | 1.52             |
| <b>VELUM® PRIME 500 SC</b>           | Fluopyram<br>(Pyridinylethylbenzamide) | 1.58                                      | 1.22             | 0.81             | 0.00             |
| <b>VELUM® PRIME 400 SC</b>           | Fluopyram<br>(Pyridinylethylbenzamide) | 0.00                                      | 0.00             | 0.00             | 0.75             |
| <b>TRIMPLE 1110 AL</b>               | 1,3-dichloropropene                    | 0.00                                      | 0.00             | 37.50            | 40.80            |
| <b>TELONE-II™ 1110AL</b>             | 1,3-dichloropropene                    | 21.82                                     | 20.52            | 4.99             | 4.08             |
| <b>RUGBY® 100ME</b>                  | Cadusafos<br>(organophosphate)         | 10.50                                     | 6.25             | 4.71             | 4.01             |
| <b>RUGBY® 100GR</b>                  | Cadusafos<br>(organophosphate)         | 2.32                                      | 0.32             | 0.27             | 0.00             |
| <b>NEMATHORIN 900EC</b>              | Fosthiazate                            | 0.00                                      | 0.00             | 0.00             | 0.00             |
| <b>NEMACUR® 400EC</b>                | Fenamiphos<br>(organophosphate)        | 1.47                                      | 1.71             | 1.77             | 1.55             |
| <b>NEMACUR® 100GR</b>                | Fenamiphos<br>(organophosphate)        | 1.02                                      | 0.49             | 0.42             | 0.40             |
| <b>MOCAP® 150GR</b>                  | Ethoprophos<br>(organophosphate)       | 1.65                                      | 2.66             | 0.39             | 0.37             |
| <b>HERBIFUME® 510GE</b>              | Metham sodium<br>(Thiocarbamate)       | 2.20                                      | 2.06             | 1.53             | 1.46             |
| <b>FENAMIPHOS 400EC</b>              | Fenamiphos<br>(organophosphate)        | 3.22                                      | 4.24             | 4.79             | 4.50             |
| <b>EDB 1800VP</b>                    | Ethylene dibromide                     | 9.86                                      | 11.22            | 5.14             | 4.81             |
| <b>DILONE 1110AL</b>                 | 1,3-dichloropropene                    | 0.00                                      | 2.97             | 1.38             | 1.31             |
| <b>DICHLOROPROPENE 1110AL</b>        | 1,3-dichloropropene                    | 0.00                                      | 0.00             | 0.00             | 0.00             |
| <b>CROP-GUARD® 900EC</b>             | Furfural (aldehyde)                    | 1.41                                      | 1.59             | 1.18             | 1.12             |
| <b>BLOCKADE™ 310SL</b>               | Oxamyl (carbamate)                     | 2.56                                      | 3.04             | 2.45             | 3.33             |
| <b>BLOCKADE™ 010GR</b>               | Oxamyl (carbamate)                     | 3.17                                      | 7.74             | 6.71             | 6.28             |
| <b>AG-FUME 510SF</b>                 | Metam sodium<br>(Thiocarbamate)        | 27.56                                     | 25.81            | 19.17            | 18.27            |
| <b>Total estimated quantity (kg)</b> |                                        | <b>1 768 950</b>                          | <b>1 888 700</b> | <b>2 543 069</b> | <b>2 668 784</b> |

Chemical nematode control has been a critical part of integrated PPN management programs. Very few innovative solutions had been developed in the last decades until the development of fluopyram was completed as a less hazardous option (See Chapter 1, paragraph 1.4.1).

Besides the use of chemicals, alternative nematode control strategies such as crop rotation, organic soil amendments or resistant, or tolerant cultivars are also implemented in many potato cropping systems (Jones *et al.*, 2017). Pressure from governments and environmentalists to reduce chemical crop protection compounds from the market has been increasing since the

1970s. The main aim is to promote the production of pesticide-free, safe food and limit the negative effects that these compounds have on the environment. Therefore, research into effective alternative control strategies has become more important (Jones, 2017). One of the earliest methods of nematode control was to ensure that organic content in the soils increased and this can be achieved by using green manures, organic manures (e.g. animal manures) and soil amendments/extracts obtained from various plant parts including leaves, stems, oils extracted from seeds and oilseed cakes (Dutta *et al.*, 2019; Peiris *et al.*, 2020).

The use of an oil extract from the leaves of the neem plant, *Azadirachta indica* A. Juss or cow manure, alone or in combination, is an example of such an eco-friendly strategy. Various studies have shown that, when used as green manure, seed oil cake and neem oil extract, these components have nematocidal (killing of nematodes) and/or nematostatic (inhibit nematode movement or reproduction) effects (Campos *et al.*, 2016; Jones, 2017). A review by Khalil (2013) showed that neem products were effective against RKN by reducing the ability of RKN second-stage juveniles (J2) to hatch and reduce their motility. However, only a few studies have been done regarding the use of neem and neem-based products in potato cropping systems to control nematodes. According to Peiris *et al.* (2020), only one out of the 136 experiments, from 85 articles used for their systematic review and meta-analysis of organic amendments used to control RKN in various crops, focussed on plant residues in potato. A more recent study done by Rossi *et al.* (2021) that focussed on the use of an organic liquid fertiliser containing neem extract showed no significant differences between any of the treatments used. Cow manure used as a solo treatment to reduce RKN and other PPN has shown variable results (Kimpinski *et al.*, 2003). Investigating neem-amended products and manure, warrants further investigation and was included in this study as an alternative and environmentally friendly option to reduce target PPN population densities.

Keeping the strategies referred to above in mind, the aims of this study were to determine whether: i) a neem oil-amended cow manure product (marketed as Kalahari 3:1) as a stand-alone but also in combination with registered nematicides, could offer a nematocidal effect against PPN under typical South African field conditions in a potato planting near Tzaneen (Limpopo province); ii) these effects are comparable to registered nematicides and iii) the neem oil-amended cow manure granules could have beneficial effects to potato tuber quality.

## **3.2 Materials and methods**

### **3.2.1 Study area**

This study was conducted near Tzaneen in the Deerpark area (23°44'23.98" S, 30°17'46.66" E), situated in the North-East of the Limpopo province of South Africa (see Figures 3.1 and 3.2). The

site selected for this study was chosen for its naturally high infestation levels of PPN, which was dominated by RKN populations - *Meloidogyne javanica* and *M. enterolobii* occurring in a mixed community in a 90:10 ratio (see paragraph 3.2.1; Figure 3.3). The soil contained 85% sand, 3% silt and 12% clay (Table 3.2). The area is known for its warm and temperate climatic conditions and high seasonal rainfall (800-1500 mm per annum). Average annual minimum and maximum temperatures ranged between 10-31 °C over the last 20 years in this area ([www.worldweatheronline.com/Tzaneen-weather-averages/Limpopo/za.aspx](http://www.worldweatheronline.com/Tzaneen-weather-averages/Limpopo/za.aspx)). The field had not been used for any agricultural practices for 15 years before this trial and the natural vegetation mostly consisted of White Buffalo (*Urochloa mosambicensis* (Hack.) Dandy.) and Natal red top (*Melinis repens* (Willd.) Zizka.) grasses and was situated adjacent to a guava (*Psidium guajava* L.) orchard. Soil collected from the site, before the onset of this study, was placed into 5-L capacity plastic buckets and planted with tomato seedlings (*Solanum lycopersicum* L., cv. Rodade), known to be susceptible to RKN (Fourie *et al.*, 2012). When the roots were lifted from the soil approximately 110 DAP, a severe RKN infestation was present, confirmed by extensive galling (Figure 3.3), making it a good site to evaluate the effects of a neem-oil amended cow manure product (Kalahari 3:1) as a solo treatment as well as in combination with various registered nematicides.



Figure 3.1: Google Maps imagery of South Africa indicating the location of the trial site located near Tzaneen in the Limpopo Lowveld (Image from Google Earth: <https://earth.google.com>).



Figure 3.2: Aerial imagery of the trial site indicated by the red rectangle (Image from Google Earth Pro; [https://earth.google.com/web/@-23.74046425,30.29656528,921.30317049a,0d,35y,-0.0005727t,-0r?utm\\_source=earth7&utm\\_campaign=vine&hl=en](https://earth.google.com/web/@-23.74046425,30.29656528,921.30317049a,0d,35y,-0.0005727t,-0r?utm_source=earth7&utm_campaign=vine&hl=en)).



Figure 3.3: Root-knot nematode galled roots of tomato plants cultivated in soil taken from the selected trial site in the Deerpark area near Tzaneen, Limpopo province of South Africa (Photos: Melissa Buys, NWU).

### 3.2.2 Trial layout and soil sampling

The trial site used was divided into 64 plots, each 5 m in width and 16 m in length.

A representative soil sample was taken across the entire field, by collecting and pooling 20 subsamples with a soil auger, in a zig-zag pattern to determine the selected soil characteristics (Table 3.1). This sample was analysed by SGS (Société Générale de Surveillance), South Africa using standard protocols.

Before planting and treatment applications commenced, a composite soil sample was collected for nematode and soil chemical and physical analysis from each plot. The composite sample per plot consisted of five subsamples which were collected using a spade. Each subsample was obtained by digging a hole of about 15 cm deep and 15 cm wide. The soil of the five subsamples was placed in the same plastic bag, mixed thoroughly and marked clearly. Sixty-four samples in total were collected from the study plot, stored in a cold room (at approximately 4-6 °C) and transported to the laboratory within two days. In the nematode laboratory, a representative sample of each plot was used for nematode analysis while the rest of the soil was used to conduct soil chemical and physical analysis for each plot.

Table 3.2: Soil physical and chemical analysis of the trial site near Deerpark area near Tzaneen, Limpopo province of South Africa where a neem-amended product as a solo treatment as well as in combination with various registered nematicides were evaluated for their efficacy against plant-parasitic nematodes.

|                                 |           |                           |          |                      |           |
|---------------------------------|-----------|---------------------------|----------|----------------------|-----------|
| <b>% Sand</b>                   | <b>85</b> | <b>% Silt</b>             | <b>3</b> | <b>% Clay</b>        | <b>12</b> |
| <b>pH (KCl)</b>                 | 7.0       | <b>K cmol(+)/kg</b>       | 0.61     | <b>Na %</b>          | 1.1       |
| <b>Exc. Acid cmol(+)/kg*</b>    | 0.00      | <b>Ca cmol(+)/kg</b>      | 4.29     | <b>Acid sat.</b>     | 0.0       |
| <b>K mg/kg</b>                  | 237       | <b>Mg cmol(+)/kg</b>      | 1.12     | <b>Base sat</b>      | 100.0     |
| <b>Ca mg/kg</b>                 | 860       | <b>Na cmol(+)/kg</b>      | 0.06     | <b>Ca:Mg</b>         | 3.82      |
| <b>Mg mg/kg</b>                 | 137       | <b>T value cmol(+)/kg</b> | 6.1      | <b>(Ca+Mg)/K</b>     | 8.93      |
| <b>Na mg/kg</b>                 | 15        | <b>K %</b>                | 10       | <b>Mg:K</b>          | 1.86      |
| <b>Density g/cm<sup>3</sup></b> | 1.401     | <b>Ca %</b>               | 70.5     | <b>Na:K</b>          | 0.11      |
| <b>S value cmol(+)/kg</b>       | 6.1       | <b>Mg %</b>               | 18.5     | <b>P-Bray1 mg/kg</b> | 23        |

\*Exchangeable acidity (KCl)

The trial consisted of 7 treatments (including chemical and the organic-based treatments) and untreated control (Table 3.3), replicated 8 times, using a randomised complete block design (Figure 3.4). The treatments included products with nematicidal or nematostatic properties of which two (fenamiphos and fluopyram a.i.) are registered on potato in South Africa (Agri-Intel, 2021). A third environmentally friendly product, Kalahari 3:1, developed and sold by Organic Fertilizer Manufacturers Botswana (OFMB), was included and consisted of a neem-oil amended cow manure granular product, commercially known and sold as Kalahari 3:1. Table 3.3 shows the information of the three products included in the trial.

The neem-oil amended cow manure product consisted of a combination of cow manure (80%), lime (10%) and carbon (10%) and is a mixture of Neem oil @ 125 L/ton (known as an organic nematode repellent), Cosmoroot @ 5 g/ton (200N, 87P, 166K g/kg – root stimulant), H85 @ 5 kg/ton (850g/g Humus (carbon)), Soilex @ 5 L/ton (64 g/L organic extracts, 63 g/L carboxylic acids – sodium neutraliser) and Mainstay Ca @ 5 L/ton (200 g/L – 42%CaO).

Table 3.3: Treatments used in the trial showing information about the product descriptions, dosage rates, crops they are registered on and manufacturers.

| Treat ment no. | Description, active ingredient and IRAC group                 | Dosage rate applied per plot                | Dose rate per ha                                                        | Type                      | Crops registered on             | Manufacturer                                     |
|----------------|---------------------------------------------------------------|---------------------------------------------|-------------------------------------------------------------------------|---------------------------|---------------------------------|--------------------------------------------------|
| 1.             | Untreated control                                             | -                                           | -                                                                       |                           |                                 |                                                  |
| 2.             | Nemacur® 400 EC, Fenamiphos @ 400 g / L, IRAC: 1B             | 200 ml/2 L water                            | 250 ml/2.5 L water/100 m plant furrow (25 L/ha for rows 1 m apart)      | Organo-phosphate          | Potato, Citrus, Tomato and more | Villa Crop Protection                            |
| 3.             | Nemacur® 400 EC + Kalahari 3:1                                | 200 ml/2 L water + 6.4 kg                   | 25 L + 800 kg                                                           |                           |                                 |                                                  |
| 4.             | Nemacur® 400 EC +Velum® Prime 400 SC                          | 200 ml/2 L water + 16 ml/2 L water          | 25 L 25 ml/2.5 L water/100 m plant furrow (2.5 L/ha for rows 1 m apart) |                           |                                 |                                                  |
| 5.             | Nemacur® 400 EC +Velum® Prime 400 SC + Kalahari 3:1           | 200 ml/2 L water + 16 ml/2 L water + 6.4 kg | 25 L 2.5 L 800 kg                                                       |                           |                                 |                                                  |
| 6.             | Velum® Prime 400 SC, Fluopyram @ 400 g / L, IRAC: 25; FRAC: 7 | 16 ml/2 L water                             | 2.5 L                                                                   | Pyridinyl ethyl benzamide | Citrus, Potato, Tobacco, Tomato | Bayer (Pty) Ltd)                                 |
| 7.             | Velum® Prime 400 SC + Kalahari 3:1                            | 16 ml/2 L water + 6.4 kg                    | 2.5 L + 800 kg                                                          |                           |                                 |                                                  |
| 8.             | Kalahari 3:1, organic fertiliser                              | 6.4 kg                                      | 800 kg                                                                  | Cow manure (80%)          | Broad spectrum                  | Organic Fertilizer Manufacturers Botswana (OFMB) |

Each plot consisted of 5 rows, planted by hand, with potato cv. Sifra, which was obtained from Legkraal Boerdery, a registered table and seed potato producer, located in Dendron, Limpopo. The planned planting date was delayed due to drought conditions and little to no water available for irrigation and potato tubers were eventually planted on the 5<sup>th</sup> of October 2018. After planting, drip irrigation (2 L/h drippers, 2 bar pressure at 30 cm spacing) was installed on each ridge. The date of initial seedling emergence was the 9<sup>th</sup> of November 2018. The plots receiving Kalahari 3:1, at a rate of 6.4 kg per plot, were treated in-furrow before tubers were placed. Nemacur® 400 EC treatments were applied using a knapsack sprayer at a rate of 200 ml/2 L water per plot over the tubers in-furrow, while the Velum® Prime 400 SC treatments were applied at a rate of 16 ml/2

L water per plot over the tubers in-furrow as per registration (Agri-Intel, 2021). After the treatments had been applied, the tubers were covered with soil and slightly ridged by hand.

| <i>Rep 1</i> | <i>Rep 2</i> | <i>Rep 3</i> | <i>Rep 4</i> | <i>Rep 5</i> | <i>Rep 6</i> | <i>Rep 7</i> | <i>Rep 8</i> |
|--------------|--------------|--------------|--------------|--------------|--------------|--------------|--------------|
| 8            | 6            | 2            | 3            | 5            | 4            | 1            | 7            |
| 4            | 1            | 5            | 8            | 7            | 3            | 6            | 2            |
| 3            | 7            | 4            | 6            | 1            | 2            | 8            | 5            |
| 1            | 5            | 3            | 2            | 8            | 6            | 7            | 4            |
| 7            | 8            | 1            | 4            | 3            | 5            | 2            | 6            |
| 5            | 2            | 7            | 1            | 6            | 8            | 4            | 3            |
| 6            | 4            | 8            | 5            | 2            | 7            | 3            | 1            |
| 2            | 3            | 6            | 7            | 4            | 1            | 5            | 8            |

Figure 3.4.: A field trial layout showing the randomisation of eight treatments consisting of two registered nematicides and a neem-oil amended cow manure granule which were evaluated singly and in combination with a registered nematicide for its efficacy against plant-parasitic nematodes in the Deerpark area near Tzaneen, Limpopo province, South Africa. Treatments were as follows: 1 = Untreated control; 2 = Nematicur 400 EC @ 200 ml/2 L water; 3 = Nematicur 400 EC @ 200 ml/2 L water + Kalahari 3:1 @ 6.4 kg/plot; 4 = Nematicur 400 EC @ 200 ml/ 2 L water + Velum Prime 400 SC @ 16 ml/ 2 L water; 5 = Nematicur 400 EC @ 200 ml/ 2 L water + Velum Prime 400 SC 16 ml/ 2 L water + Kalahari 3:1 @ 6.4 kg/plot; 6 = Velum Prime 400 SC @ 16 ml/2L water; 7 = Velum Prime 400 SC @ 16 ml/ 2 L water + Kalahari 3:1 @ 6.4 kg/plot; 8 = Kalahari 3:1 @ 6.4 kg/plot (Illustration by Melissa Buys, NWU).

### 3.2.3 Nematode sampling: root and soil

At 10 weeks after plant emergence and at harvest (19 weeks after emergence), soil and root samples were collected again for nematode analyses. Soil samples were taken from each plot as described above (Paragraph 3.2.2) and put in labelled plastic bags, while root samples were

collected from three plants per plot. Sampling dates are indicated in Table 3.4. These plants were uprooted and the root systems were cut above ground level and placed in the same plastic bag containing the soil of the corresponding plot. Samples were placed in a cold room (4-6 °C) before being transported to the laboratory of the Agricultural Research Council-Tropical and Subtropical Crops (ARC-TSC, Mbombela, Mpumalanga province) where extractions were done to isolate nematodes from roots and soil.

Table 3.4: Sampling intervals of soil and roots for nematode analyses from the trial site situated on Nyalani Estates, Deerpark near Tzaneen.

| Sample timing | Dates           | Soil | Roots |
|---------------|-----------------|------|-------|
| Pre-plant     | 5 October 2018  | Yes  | No    |
| 10 WAE        | 18 January 2018 | Yes  | Yes   |
| Harvest       | 25 March 2019   | Yes  | Yes   |

### 3.2.4 Long-term precipitation and temperature data

Since the farm, on which the trial site is located, had been managed under the current owner, all precipitation events were recorded and compared to the long-term average of the area. This data shows that, during the trial period, severe drought conditions were prevalent, which started in the 2014/15 season (Figure 3.5). During the 2016/17 season, the area experienced a good rainfall season (934 mm) compared to 443 mm in the 2014/15 season and 637 mm in the 2015/16 season, which is extremely low for this region. But unfortunately, this did not increase the availability of irrigation water in the natural dams on the farm and the amount of water obtained from the borehole remained low and inconsistent, indicating that the underground water reserves did not recover. After excellent precipitation in the 2020/21 season (1073 mm), which was close to the normal rainfall figures, conditions improved which had a positive effect on the locally available underground water resources, as seen by the amount and stability of water obtained from the borehole on the farm. Figure 3.5 clearly shows a reduction in summer rainfall after 2014. The long-term temperature data is presented in Figure 3.6 showing a spike in average maximum temperatures in 2014 and again between December 2015 - January 2016. The temperatures were unlikely to have as big an impact on the outcome of the trial as the reduced amount of rainfall had.

The trial was planted in October 2018, when the seasonal (2018/2019) average was only 68.07% of the local average precipitation over 11 years. Before the first dry season (2014/15) was experienced, the local average rainfall was 1148.33 mm per season.

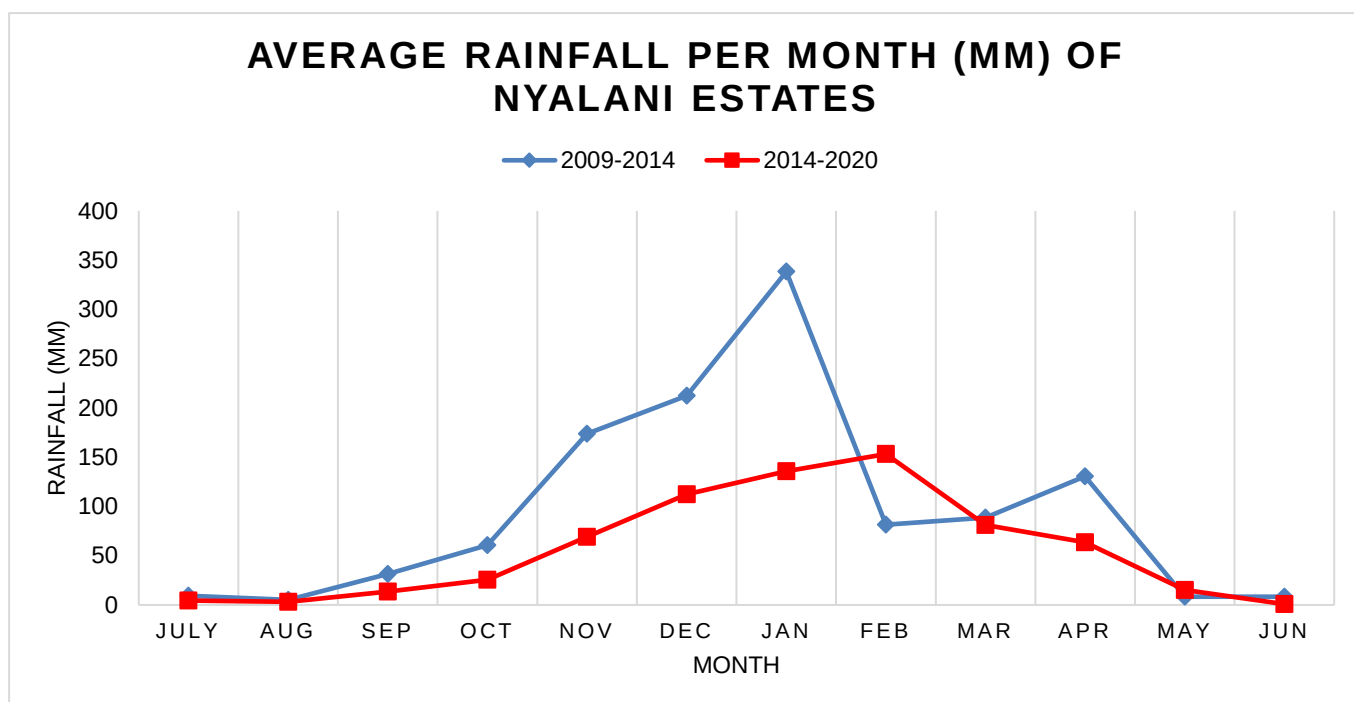


Figure 3.5: Long-term precipitation (in mm) record of Nyalani estates, where the potato-nematode study was done, from July 2008-June 2021, Deerpark near Tzaneen where this study was conducted.

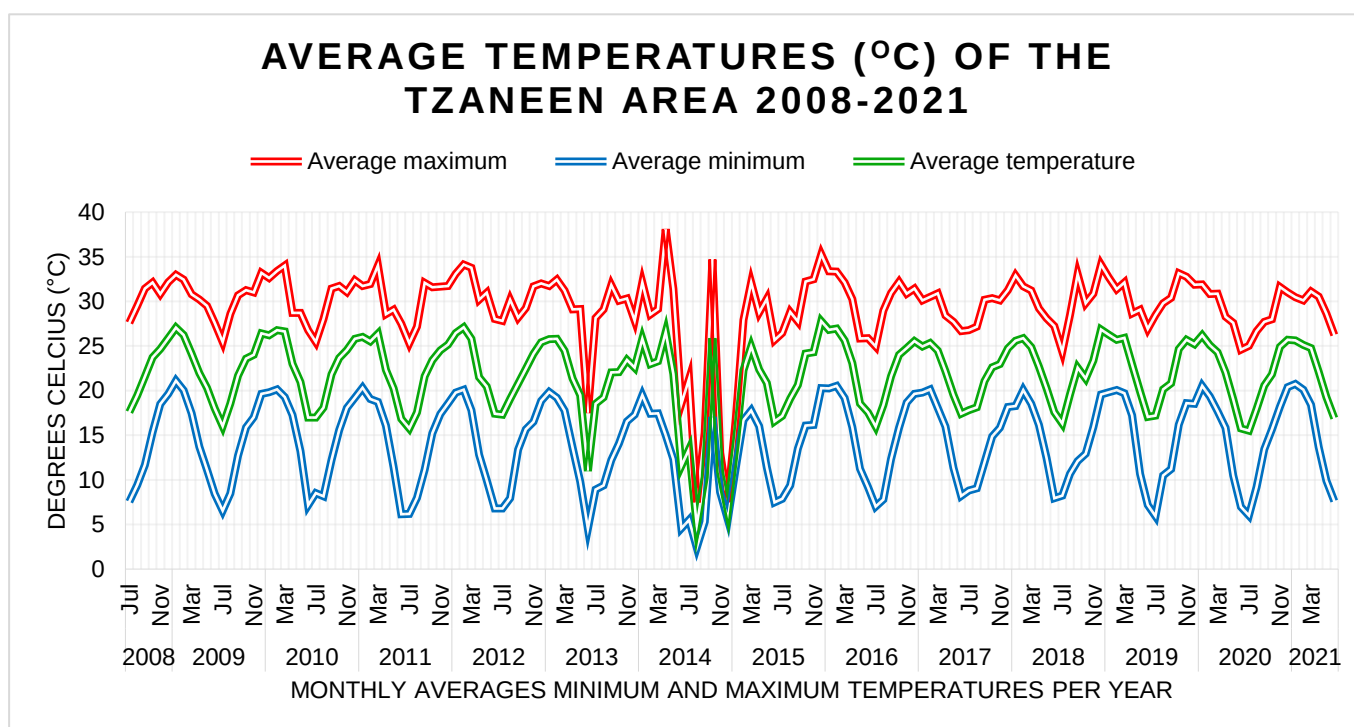


Figure 3.6: Long-term temperature record of the Tzaneen-Letaba area, in which Nyalani Estates, Deerpark where the potato-nematode study was done is situated, from July 2008-June 2021 (credit to ARC-SCW).

### **3.2.5 Nematode analyses**

#### **3.2.5.1 Soil**

Nematodes were extracted from soil samples using the decanting and sieving methods described by Hooper *et al.* (2005) and Marais *et al.* (2017), with some adaptations being made as described below.

Two hundred and fifty millilitres of soil from each sample was soaked in tap water and washed through a coarse meshed 2 mm aperture sieve into a bucket to remove plant tissues and rocks from the soil. The bucket was filled up to 5 L with tap water and the soil was mixed well. The mixture was then left to stand for 30 seconds. The supernatant was decanted through stacked 45- $\mu\text{m}$ -aperture and 25- $\mu\text{m}$ -aperture sieves and the sediment left in the bucket. The nematodes caught on the sieves were washed with distilled water into a beaker. The process was repeated twice with shorter settling times (20 seconds and 10 seconds) using the sediment in the bucket. The residues of each washing process were collected in a beaker. The contents of the beakers were thoroughly mixed and divided into 100 ml centrifuge tubes to be centrifuged. Kaolin (5 ml) was added to each tube, mixed and centrifuged at 1800 g for 4 minutes. The supernatant was discarded and a sucrose solution (specific gravity of 1.15 g cm<sup>3</sup>) was added to each tube and mixed well and centrifuged at 1800 g for 3 minutes. The nematodes contained in the supernatant were decanted through a 25- $\mu\text{m}$ -aperture sieve and washed with distilled water to remove the sucrose solution. The residues were then washed into a beaker with distilled water for counting and identification (Marais *et al.*, 2017).

#### **3.2.5.2 Roots**

Nematodes were extracted from roots using the adapted sugar flotation method described by Hooper *et al.* (2005) and Marais *et al.* (2017) with adaptations being made as described below.

The root pieces obtained per composite samples were collected from the plastic bag of each plot and cut into 2-5 mm pieces. Subsequently, 30 g was randomly selected and placed in a blender with 250 ml tap water and macerated at medium speed for 30-45 seconds, releasing the nematodes from the plant material. The water suspension was decanted over nested sieves with the aperture sizes as follows: 1-mm, 150- $\mu\text{m}$ , 45- $\mu\text{m}$ , 38- $\mu\text{m}$  and 25- $\mu\text{m}$  apertures. The plant tissues on the 1mm aperture sieve were thoroughly washed with tap water and then discarded. The residues on the stacked sieves were collected into 50 ml centrifugal tubes. Kaolin was added to each tube and the contents mixed well. The tubes were then centrifuged at 1800 g for 4 minutes. The supernatant was removed and a sucrose solution (specific gravity of 1.15 g cm<sup>3</sup>) was added to each tube and mixed well. The tubes were centrifuged again at 1800 g for 3 minutes. The nematodes contained in the supernatant were decanted through a 25- $\mu\text{m}$  aperture sieve and

washed with distilled water to remove the sucrose solution. The residues were then washed into a beaker with distilled water for counting and identification (Hooper *et al.*, 2005; Marais *et al.*, 2017).

Kaolin is a very useful compound to use in nematode extractions due to its small, flat shape. This clay mineral has a specific gravity of 2.6 g/cm<sup>3</sup>, which is higher than the gravity of nematodes, but the shape of these 2-3µm sized particles means that kaolin sinks to the bottom of the centrifuge tube slower than nematodes. The kaolin forms a layer over the nematode containing sediment in the centrifuge tube, sealing it off from the supernatant that is discarded. The kaolin layer is then broken through thorough stirring after the sugar solution is added, to bring the nematodes into suspension. During the sugar centrifugation, kaolin precipitates which prevents the sediment from remixing with the supernatant, when the sugar solution that contains the suspended nematodes is decanted (Marais *et al.*, 2017).

### **3.2.6 Counting of nematodes**

#### **3.2.6.1 Plant-parasitic and beneficial soil nematodes**

The processed soil and root samples were each made up to 100 ml with tap water while being stirred continuously on a magnetic stirrer. A pipette was used to draw up 1 ml liquid from the sample and placed onto a 1 ml Peters slide to count each sample (Marais *et al.*, 2017). Plant-parasitic nematodes were counted to genus level while NPN were counted to trophic level with the assistance of Dr. Mieke Daneel at ARC-TSC and Prof. Driekie Fourie from the NWU.

#### **3.2.7 Damage index of potato tubers**

Visual evaluations of 50 tubers from each plot were done according to the galling scale used by Taylor and Sasser (1978) to determine the severity of RKN damage to the harvest. Table 3.5 describes the galling scale.

Table 3.5: Potato tuber galling scale (Taylor & Sasser, 1978) used to grade potato tubers according to damage caused by RKN parasitism.

| Galling index | No of galls per tuber |
|---------------|-----------------------|
| 0             | 0                     |
| 1             | 1-2                   |
| 2             | 3-10                  |
| 3             | 11-30                 |
| 4             | 31-100                |
| 5             | >100                  |

Unfortunately, due to lack of sufficient rain, the crop was diminished and yield data compromised. Therefore, all the tubers sampled from each plot were rated using the galling index indicated above with focus placed on the latter and nematode population densities. The decision was then made to focus on nematode populations and the damage index obtained from the tubers harvested and not to include yield data.

### 3.2.8 Statistical Analysis

Data obtained from the nematode counts was subjected to One-way ANOVA (Statistica 14.0.0) to compare treatments with one another per sampling interval. Data was transformed  $\log_{10}(x+1)$  before being subjected to ANOVA and Repeated Measures ANOVA, using the program Statistica 14.0.0 ([www.tibco.com](http://www.tibco.com)). The level of significance was set at  $P<0.05$ , using the Tukey HSD Test.

The percentage RKN infection on tubers for each plot was calculated from the galling index using the Townsend & Heuberger, 1943 formula (Ünal *et al.*, 2019):

$$\% \text{ Infection} = \frac{\sum(n \times v)}{iN} = 100$$

n = number of tubers per category

v = numerical value of each category

i = highest category value

N = total number of tubers in sample/plot

The average percentage for each treatment was calculated and used to calculate the percentage efficacy of each treatment against RKN tuber damage using the ABBOT calculation (Abbott, 1987):

$$\% \text{ Efficacy} = \frac{C-T}{C} \times 100$$

C = percentage infestation in the untreated plots

T = percentage infestation in the treated plots

The data obtained (% RKN damage on tubers) were then subjected to one-way ANOVA (Statistica 14.0.0).

### **3.3 Results**

#### **3.3.1 Plant-parasitic nematode data from 250 ml soil**

Plant-parasitic nematodes observed in the trial included individuals belonging to the genera *Meloidogyne*, *Pratylenchus*, *Helicotylenchus* (Steiner, 1945), *Paratrichodorus* (Siddiqi, 1974), *Tylenchorhynchus* (Cobb, 1913), *Rotylenchulus* (Linford & Oliveira, 1940), *Xiphinema* (Cobb, 1913) and *Hemicycliophora* (de Man. 1921) as well as from the family Criconematidae (Taylor, 1936). However, only data for *Meloidogyne*, *Pratylenchus*, Hoplolaimidae (Filipjev, 1934) and Criconematidae is discussed, as the other PPN population densities were detected in low numbers (<100 individuals/250 ml soil during all sampling intervals).

##### **3.3.1.1 Root-knot nematodes**

No significant differences were evident among the treatments per sampling interval, nor did a significant interaction exist for treatments x sampling times (Table 3.6) indicating that RKN population densities were similar for all treatments and sampling times although it did increase over the sampling times.

Population densities for RKN in 250 ml soil samples were low before the commencement of treatment applications and planting activities (Pre-plant sampling interval), ranging from zero to 13 (Table 3.6). At 10 weeks after crop emergence (WAE; Sampling interval 2), the average RKN population densities slightly increased (ranging from zero to 44/250 ml soil). This was evident for the untreated control, treatment 4 and 5 plots, but remained unchanged in treatment 8 plots. By the harvest sampling date (19 WAE; Sampling interval 3), all the treatments showed a substantial increase in RKN counts but still did not differ significantly from each other.

Table 3.6: Root-knot nematode (*Meloidogyne*) second-stage juvenile (J2) population densities in 250 ml soil for three sampling intervals. (Significance level for Repeated measures ANOVA in capital letters indicating that different letters indicate significant differences across rows for sampling intervals; different small letters per column indicate significant differences among treatments per individual sampling interval).

| Treatment                                           | Treatment name                                                | Sampling interval 1:<br>Pre-plant     | Sampling interval 2:<br>10 WAE | Sampling interval 3:<br>19 WAE<br>(Harvest) |
|-----------------------------------------------------|---------------------------------------------------------------|---------------------------------------|--------------------------------|---------------------------------------------|
| 1                                                   | Untreated control                                             | 0.21 <sup>1</sup> (6) <sup>2</sup> aA | 0.73 (44) aA                   | 2.23 (994) aAB                              |
| 2                                                   | Nemacur® 400 EC, Fenamiphos @ 400 g / L, IRAC: 1B             | 0.0 (0) aA                            | 0.0 (0) aA                     | 0.79 (56) aA                                |
| 3                                                   | Nemacur® 400 EC + Kalahari 3:1                                | 0.0 (0) aA                            | 0.0 (0) aA                     | 1.91 (363) aA                               |
| 4                                                   | Nemacur® 400 EC +Velum® Prime 400 SC                          | 0.25 (13) aA                          | 0.21 (6) aA                    | 0.82 (363) aA                               |
| 5                                                   | Nemacur® 400 EC +Velum® Prime 400 SC + Kalahari 3:1           | 0.25 (13) aA                          | 0.32 (44) aA                   | 1.57 (338) aA                               |
| 6                                                   | Velum® Prime 400 SC, Fluopyram @ 400 g / L, IRAC: 25; FRAC: 7 | 0.0 (0) aA                            | 0.0 (0) aA                     | 1.51 (1081) aAB                             |
| 7                                                   | Velum® Prime 400 SC + Kalahari 3:1                            | 0.0 (0) aA                            | 0.0 (0) aA                     | 1.97 (500) aAB                              |
| 8                                                   | Kalahari 3:1, organic fertiliser                              | 0.21 (6) aA                           | 0.21 (6) aA                    | 2.61(1650) aB                               |
| <b>F-ratio:</b>                                     |                                                               | 0.575                                 | 1.588                          | 1.919                                       |
| <b>P-value:</b>                                     |                                                               | 0.773                                 | 0.158                          | 0.083                                       |
| <b>Interaction: Treatments x Sampling intervals</b> |                                                               |                                       |                                |                                             |
| <b>F-ratio:</b>                                     |                                                               | 1.51                                  |                                |                                             |
| <b>P-value:</b>                                     |                                                               | 0.21                                  |                                |                                             |

<sup>1</sup>Log-transformed mean of nematode population density; <sup>2</sup>Real means of nematode population density.

### 3.3.1.2 Lesion nematodes

Statistically significant differences were evident between the untreated control (treatment 1) and treatments 2 and 5 at the 10 WAE sampling date (sampling interval 2) only (Table 3.7). No significant differences were evident among the treatments at the pre-plant or harvest sampling dates (sampling intervals 1 and 3), nor did any significant interactions exist for treatment x sampling times. This indicates that LN population densities were similar for treatments and sampling times.

Population numbers for LN in 250 ml soil samples were fairly uniform in all the plots at the pre-plant sampling date (>100 but <200 individuals/250 ml soil). At 10 WAE (sampling interval 2), the population counts for the untreated plots differed significantly from treatments 2-5, which all showed reduced LN population densities at this sampling date. By the harvest sampling date (19 WAE; sampling interval 3), most of the treatments showed an increase in population numbers when compared to the 10 WAE sampling interval, but not to the pre-plant sampling interval.

Table 3.7: Lesion nematode (*Pratylenchus*) population densities in 250 ml soil for three sampling intervals. (Significance level for Repeated measures ANOVA in capital letters; different letters indicate significant differences across rows for sampling intervals; different small letters per column indicate significant differences among treatments per individual sampling interval).

| Treatment                                           | Treatment name                                                | Sampling interval 1:<br>Pre-plant       | Sampling interval 2:<br>10 WAE | Sampling interval 3:<br>19 WAE (Harvest) |
|-----------------------------------------------------|---------------------------------------------------------------|-----------------------------------------|--------------------------------|------------------------------------------|
| 1                                                   | Untreated control                                             | 1.13 <sup>1</sup> (106) <sup>2</sup> aA | 1.86 (238) bA                  | 1.30 (200) aA                            |
| 2                                                   | Nemacur® 400 EC, Fenamiphos @ 400 g / L, IRAC: 1B             | 1.34 (119) aA                           | 0.21 (6) aA                    | 1.56 (100) aA                            |
| 3                                                   | Nemacur® 400 EC + Kalahari 3:1                                | 1.36 (131) aA                           | 0.43 (13) abA                  | 0.60 (75) aA                             |
| 4                                                   | Nemacur® 400 EC +Velum® Prime 400 SC                          | 1.15 (106) aA                           | 0.51 (38) abA                  | 1.13 (119) aA                            |
| 5                                                   | Nemacur® 400 EC +Velum® Prime 400 SC + Kalahari 3:1           | 1.39 (138) aA                           | 0.32 (44) aA                   | 1.52 (94) aA                             |
| 6                                                   | Velum® Prime 400 SC, Fluopyram @ 400 g / L, IRAC: 25; FRAC: 7 | 1.63 (163) aA                           | 0.83 (81) abA                  | 1.11 (88) aA                             |
| 7                                                   | Velum® Prime 400 SC + Kalahari 3:1                            | 1.63 (181) aA                           | 1.01 (56) abA                  | 0.84 (69) aA                             |
| 8                                                   | Kalahari 3:1, organic fertiliser                              | 0.89 (100) aA                           | 0.52 (44) abA                  | 0.27 (19) aA                             |
| F-ratio:                                            |                                                               | 0.377                                   | 2.377                          | 1.284                                    |
| P-value:                                            |                                                               | 0.912                                   | <b>0.034</b>                   | 0.275                                    |
| <b>Interaction: Treatments x Sampling intervals</b> |                                                               |                                         |                                |                                          |
| F-ratio:                                            |                                                               |                                         | 1.375                          |                                          |
| P-value:                                            |                                                               |                                         | 0.177                          |                                          |

<sup>1</sup>Log-transformed mean of nematode population density; <sup>2</sup>Real means of nematode population density.

### 3.3.1.3 Spiral nematodes

No significant differences were evident among the treatments per sampling interval, nor did a significant interaction exist for treatments x sampling times (Table 3.8) indicating that spiral nematode population densities were fairly similar for all treatments and sampling times.

Population densities for spiral nematodes in 250 ml soil samples were high before the commencement of treatment applications and planting activities (Pre-plant sampling interval), ranging from 219 to 1163. At 10 WAE (Sampling interval 2), the average spiral nematode population densities decreased in all treatments and the untreated control (ranging from six to 200/250 ml soil). By the harvest sampling date (19 WAE; Sampling interval 3), most of the treatments showed a slight decline in spiral nematode counts but did not differ significantly from each other.

Table 3.8: Spiral nematode (Hoplolaimidae) population densities in 250 ml soil for three sampling intervals. (Significance level for Repeated measures ANOVA in capital letters; different letters indicate significant differences across rows for sampling intervals; different small letters per column indicate significant differences among treatments per individual sampling interval).

| Treatment                                           | Treatment name                                                | Sampling interval 1:<br>Pre-plant        | Sampling interval 2:<br>10 WAE | Sampling interval 3:<br>19 WAE<br>(Harvest) |
|-----------------------------------------------------|---------------------------------------------------------------|------------------------------------------|--------------------------------|---------------------------------------------|
| 1                                                   | Untreated control                                             | 1.60 <sup>1</sup> (350) <sup>2</sup> aAB | 1.72 (194) aAB                 | 1.27 (106) aAB                              |
| 2                                                   | Nemacur® 400 EC, Fenamiphos @ 400 g / L, IRAC: 1B             | 1.52 (219) aAB                           | 1.04 (69) aAB                  | 0.54 (38) aAB                               |
| 3                                                   | Nemacur® 400 EC + Kalahari 3:1                                | 1.50 (407) aAB                           | 1.17 (188) aAB                 | 0.50 (31) aAB                               |
| 4                                                   | Nemacur® 400 EC +Velum® Prime 400 SC                          | 2.17 (963) aAB                           | 0.21 (6) aA                    | 0.80 (69) aAB                               |
| 5                                                   | Nemacur® 400 EC +Velum® Prime 400 SC + Kalahari 3:1           | 1.87 (269) aAB                           | 0.79 (106) aAB                 | 0.90 (94) aAB                               |
| 6                                                   | Velum® Prime 400 SC, Fluopyram @ 400 g / L, IRAC: 25; FRAC: 7 | 1.75 (725) aAB                           | 1.20 (100) aAB                 | 0.95 (181) aAB                              |
| 7                                                   | Velum® Prime 400 SC + Kalahari 3:1                            | 2.56 (1163) aB                           | 1.49 (200) aAB                 | 1.78 (200) aAB                              |
| 8                                                   | Kalahari 3:1, organic fertiliser                              | 2.21 (800) aAB                           | 1.35 (169) aAB                 | 1.03 (256) aAB                              |
| <b>F-ratio:</b>                                     |                                                               | 0.648                                    | 1.373                          | 0.975                                       |
| <b>P-value:</b>                                     |                                                               | 0.714                                    | 0.235                          | 0.458                                       |
| <b>Interaction: Treatments x Sampling intervals</b> |                                                               |                                          |                                |                                             |
| <b>F-ratio:</b>                                     |                                                               | 0.890                                    |                                |                                             |
| <b>P-value:</b>                                     |                                                               | 0.569                                    |                                |                                             |

<sup>1</sup>Log-transformed mean of nematode population density; <sup>2</sup>Real means of nematode population density.

#### 3.3.1.4 Ring nematodes

No significant differences were evident among the treatments per sampling interval (Table 3.9). The only significant difference evident for interactions between treatments x sampling times was between treatment 1 at pre-plant sampling versus treatment 7 at 10 WAE (sampling interval 2). This indicates that, overall, the ring nematode population densities were similar between all treatments and sampling intervals.

Population densities for ring nematodes in 250 ml soil samples generally showed a decrease in population numbers at 10 WAE (sampling interval 2). Only treatment 1 showed a steady increase in ring nematode population densities over time, although this increase was not statistically significant.

Table 3.9: Ring nematode (Criconeematidae) population densities in 250 ml soil for three sampling intervals. (Significance level for Repeated measures ANOVA in capital letters; different letters indicate significant differences across rows for sampling intervals; different small letters per column indicate significant differences among treatments per individual sampling interval).

| Treatment                                           | Treatment name                                                | Sampling interval 1:<br>Pre-plant    | Sampling interval 2:<br>10 WAE | Sampling interval 3:<br>19 WAE<br>(Harvest) |
|-----------------------------------------------------|---------------------------------------------------------------|--------------------------------------|--------------------------------|---------------------------------------------|
| 1                                                   | Untreated control                                             | 0.0 <sup>1</sup> (0) <sup>2</sup> aA | 1.74 (194) aAB                 | 1.92 (306) aAB                              |
| 2                                                   | Nemacur® 400 EC, Fenamiphos @ 400 g / L, IRAC: 1B             | 0.97 (169) aAB                       | 1.12 (106) aAB                 | 1.06 (76) aAB                               |
| 3                                                   | Nemacur® 400 EC + Kalahari 3:1                                | 0.68 (25) aAB                        | 0.59 (56) aAB                  | 0.87 (88) aAB                               |
| 4                                                   | Nemacur® 400 EC +Velum® Prime 400 SC                          | 1.09 (94) aAB                        | 0.77 (50) aAB                  | 1.22 (163) aAB                              |
| 5                                                   | Nemacur® 400 EC +Velum® Prime 400 SC + Kalahari 3:1           | 1.35 (131) aAB                       | 0.77 (56) aAB                  | 0.65 (113) aAB                              |
| 6                                                   | Velum® Prime 400 SC, Fluopyram @ 400 g / L, IRAC: 25; FRAC: 7 | 0.95 (44) aAB                        | 0.95 (181) aAB                 | 0.93 (119) aAB                              |
| 7                                                   | Velum® Prime 400 SC + Kalahari 3:1                            | 0.93 (288) aAB                       | 2.21 (206) aB                  | 0.55 (63) aAB                               |
| 8                                                   | Kalahari 3:1, organic fertiliser                              | 1.64 (481) aAB                       | 0.86 (75) aAB                  | 0.99 (294) aAB                              |
| F-ratio:                                            |                                                               | 1.439                                | 2.091                          | 0.938                                       |
| P-value:                                            |                                                               | 0.208                                | 0.059                          | 0.485                                       |
| <b>Interaction: Treatments x Sampling intervals</b> |                                                               |                                      |                                |                                             |
| F-ratio:                                            |                                                               |                                      | 2.238                          |                                             |
| P-value:                                            |                                                               |                                      | <b>0.010</b>                   |                                             |

<sup>1</sup>Log-transformed mean of nematode population density; <sup>2</sup>Real means of nematode population density.

### 3.3.2 Plant-parasitic nematode data from 30 g roots

Only *Meloidogyne* (J2 and females), *Pratylenchus* and *Hoplolaimidae* population densities, as well as total PPN eggs found in the roots, are discussed since these groups dominated. Other PPN population densities detected were at most only present in 4 plots in the entire trial site and only during one of the 2 sampling dates, in numbers <417/30 g roots. Root data only covers the 10 WAE and harvest sampling dates, as there were no roots in the soil at the pre-plant sampling.

#### 3.3.2.1 Root-knot nematodes

No significant differences were evident among the treatments per sampling interval, nor did a significant interaction exist for treatments x sampling times (Table 3.10) indicating that RKN population densities in 30 g roots were fairly similar for all treatments and sampling times, although it did increase over the sampling times.

Population densities for RKN in 30 g root samples varied between zero to 688/30 g roots at 10 WAE (sampling interval 2). By the harvest sampling date (19 WAE; Sampling interval 3), most of the treatments showed an increase in RKN counts but did not differ significantly from each other.

The increase was evident for all the treatments, except treatments 2 and 4 where population densities declined when compared to the 10 WAE sampling interval.

Table 3.10: Root-knot nematode (*Meloidogyne*) population densities (all life stages, except eggs) in 30 g roots for two sampling intervals. (Significance level for Repeated measures ANOVA in capital letters; different letters indicate significant differences across rows for sampling intervals; different small letters per column indicate significant differences among treatments per individual sampling interval).

| Treatment                                           | Treatment name                                                | Sampling interval 2:<br>10 WAE          | Sampling interval 3:<br>19 WAE (Harvest) |
|-----------------------------------------------------|---------------------------------------------------------------|-----------------------------------------|------------------------------------------|
| 1                                                   | Untreated control                                             | 0.99 <sup>1</sup> (171) <sup>2</sup> aA | 1.54 (915) aA                            |
| 2                                                   | Nemacur® 400 EC, Fenamiphos @ 400 g / L, IRAC: 1B             | 0.66 (256) aA                           | 1.42 (137) aA                            |
| 3                                                   | Nemacur® 400 EC + Kalahari 3:1                                | 0.0 (0) aA                              | 0.82 (97) aA                             |
| 4                                                   | Nemacur® 400 EC +Velum® Prime 400 SC                          | 0.90 (688) aA                           | 0.31 (38) aA                             |
| 5                                                   | Nemacur® 400 EC +Velum® Prime 400 SC + Kalahari 3:1           | 0.36 (103) aA                           | 0.90 (114) aA                            |
| 6                                                   | Velum® Prime 400 SC, Fluopyram @ 400 g / L, IRAC: 25; FRAC: 7 | 1.25 (203) aA                           | 1.11 (1201) aA                           |
| 7                                                   | Velum® Prime 400 SC + Kalahari 3:1                            | 0.0 (0) aA                              | 0.64 (143) aA                            |
| 8                                                   | Kalahari 3:1, organic fertiliser                              | 0.90 (101) aA                           | 1.60 (1025) aA                           |
| F-ratio:                                            |                                                               | 1.415                                   | 0.890                                    |
| P-value:                                            |                                                               | 0.218                                   | 0.521                                    |
| <b>Interaction: Treatments x Sampling intervals</b> |                                                               |                                         |                                          |
| F-ratio:                                            |                                                               | 0.642                                   |                                          |
| P-value:                                            |                                                               | 0.720                                   |                                          |

<sup>1</sup>Log-transformed mean of nematode population density; <sup>2</sup>Real means of nematode population density.

### 3.3.2.2 Lesion nematodes

No significant differences were evident among the treatments per sampling interval, nor did a significant interaction exist for treatments x sampling times (Table 3.11) indicating that LN population densities in 30 g roots were fairly similar for all treatments and sampling times although it did decrease for most treatments over the sampling times.

Population densities for LN in 30 g root samples were high ranging between 168 to 933/30 g roots at 10 WAE (sampling interval 2). By the harvest sampling date (19 WAE; Sampling interval 3), most of the treatments showed a decrease in LN counts but still did not differ significantly from each other. This was evident for all the treatments, except treatments 3, 4 and 7 where population densities increased slightly.

Table 3.11: Lesion nematode (*Pratylenchus*) population densities in 30 g roots for two sampling intervals. (Significance level for Repeated measures ANOVA in capital letters; different letters indicate significant differences across rows for sampling intervals; different small letters per column indicate significant differences among treatments per individual sampling interval).

| Treatment                                           | Treatment name                                                | Sampling interval 2:<br>10 WAE          | Sampling interval 3:<br>19 WAE (Harvest) |
|-----------------------------------------------------|---------------------------------------------------------------|-----------------------------------------|------------------------------------------|
| 1                                                   | Untreated control                                             | 2.10 <sup>1</sup> (933) <sup>2</sup> aA | 1.33 (414) aA                            |
| 2                                                   | Nemacur® 400 EC, Fenamiphos @ 400 g / L, IRAC: 1B             | 1.50 (336) aA                           | 0.86 (205) aA                            |
| 3                                                   | Nemacur® 400 EC + Kalahari 3:1                                | 1.25 (183) aA                           | 0.95 (196) aA                            |
| 4                                                   | Nemacur® 400 EC +Velum® Prime 400 SC                          | 0.96 (181) aA                           | 0.91 (192) aA                            |
| 5                                                   | Nemacur® 400 EC +Velum® Prime 400 SC + Kalahari 3:1           | 1.47 (247) aA                           | 0.58 (55) aA                             |
| 6                                                   | Velum® Prime 400 SC, Fluopyram @ 400 g / L, IRAC: 25; FRAC: 7 | 0.99 (171) aA                           | 0.33 (57) aA                             |
| 7                                                   | Velum® Prime 400 SC + Kalahari 3:1                            | 0.98 (168) aA                           | 0.90 (214) aA                            |
| 8                                                   | Kalahari 3:1, organic fertiliser                              | 2.09 (931) aA                           | 0.91 (218) aA                            |
| F-ratio:                                            |                                                               | 0.967                                   | 0.433                                    |
| P-value:                                            |                                                               | 0.464                                   | 0.877                                    |
| <b>Interaction: Treatments x Sampling intervals</b> |                                                               |                                         |                                          |
| F-ratio:                                            |                                                               | 0.466                                   |                                          |
| P-value:                                            |                                                               | 0.855                                   |                                          |

<sup>1</sup>Log-transformed mean of nematode population density; <sup>2</sup>Real means of nematode population density.

### 3.3.2.3 Spiral nematodes

No significant differences were evident among the treatments per sampling interval, nor did a significant interaction exist for treatments x sampling times (Table 3.12) indicating that spiral nematode population densities in 30 g roots were similar for all treatments and sampling times, although it did increase over the sampling times in some treatments.

Population densities for spiral nematodes in 30 g root samples were low, ranging from zero to 8/30 g roots at 10 WAE (sampling interval 2). By the harvest sampling date (19 WAE; Sampling interval 3), only treatments 2, 4, 6 and 7 showed a slight increase in population densities, although the numbers in all treatments remained very low, from 0 to 63/30 g root and did not differ significantly among the treatments.

Table 3.12: Spiral nematode (Hoplolaimidae) population densities in 30 g roots for two sampling intervals. (Significance level for Repeated measures ANOVA in capital letters; different letters indicate significant differences across rows for sampling intervals; different small letters per column indicate significant differences among treatments per individual sampling interval).

| Treatment                                           | Treatment name                                                | Sampling interval 2:<br>10 WAE       | Sampling interval 3:<br>19 WAE (Harvest) |
|-----------------------------------------------------|---------------------------------------------------------------|--------------------------------------|------------------------------------------|
| 1                                                   | Untreated control                                             | 0.0 <sup>1</sup> (0) <sup>2</sup> aA | 0.0 (0) aA                               |
| 2                                                   | Nemacur® 400 EC, Fenamiphos @ 400 g / L, IRAC: 1B             | 0.0 (0) aA                           | 0.24 (10) aA                             |
| 3                                                   | Nemacur® 400 EC + Kalahari 3:1                                | 0.0 (0) aA                           | 0.0 (0) aA                               |
| 4                                                   | Nemacur® 400 EC +Velum® Prime 400 SC                          | 0.0 (0) aA                           | 0.24 (12) aA                             |
| 5                                                   | Nemacur® 400 EC +Velum® Prime 400 SC + Kalahari 3:1           | 0.0 (0) aA                           | 0.0 (0) aA                               |
| 6                                                   | Velum® Prime 400 SC, Fluopyram @ 400 g / L, IRAC: 25; FRAC: 7 | 0.22 (8) aA                          | 0.32 (54) aA                             |
| 7                                                   | Velum® Prime 400 SC + Kalahari 3:1                            | 0.0 (0) aA                           | 0.34 (63) aA                             |
| 8                                                   | Kalahari 3:1, organic fertiliser                              | 0.0 (0) aA                           | 0.0 (0) aA                               |
| F-ratio:                                            |                                                               | 1.000                                | 0.585                                    |
| P-value:                                            |                                                               | 0.441                                | 0.765                                    |
| <b>Interaction: Treatments x Sampling intervals</b> |                                                               |                                      |                                          |
| F-ratio:                                            |                                                               | 0.381                                |                                          |
| P-value:                                            |                                                               | 0.910                                |                                          |

<sup>1</sup>Log-transformed mean of nematode population density; <sup>2</sup>Real means of nematode population density.

### 3.3.2.4 Plant-parasitic nematode eggs

Population densities of the plant-parasitic eggs extracted from roots were substantially higher than those of the vermiform nematodes for RKN, LN and spiral nematodes per 30 g roots discussed above (Tables 3.10, 3.11 and 3.12). Some significant differences were evident among some of the treatments per sampling interval and a significant interaction existed for treatments x sampling times (Table 3.13) indicating that the number of PPN eggs in 30 g roots differed for some treatments and sampling times. The number of PPN eggs increased for all treatments over the sampling times.

Plant-parasitic egg numbers in 30 g root samples varied between six to 4238/30 g roots at 10 WAE (sampling interval 2). The untreated control (treatment 1) differed significantly from all the treatments, except treatments 4 and 8. By the harvest sampling date (19 WAE; Sampling interval 3), most of the treatments showed an increase in PPN egg counts, with treatment 8 differing significantly from treatment 7 but not from the other treatments. This increase was evident for all the treatments, except treatment 4 where PPN egg counts declined.

Table 3.13: Plant-parasitic nematode egg densities in 30 g roots for two sampling intervals. (Significance level for Repeated measures ANOVA in capital letters; different letters indicate significant differences across rows for sampling intervals; different small letters per column indicate significant differences among treatments per individual sampling interval).

| Treatment                                           | Treatment name                                                | Sampling interval 2:<br>10 WAE | Sampling interval 3:<br>19 WAE (Harvest) |
|-----------------------------------------------------|---------------------------------------------------------------|--------------------------------|------------------------------------------|
| 1                                                   | Untreated control                                             | 2.52 (1288) bBCD               | 3.57 (14327) abCD                        |
| 2                                                   | Nemacur® 400 EC, Fenamiphos @ 400 g / L, IRAC: 1B             | 0.38 (164) aA                  | 2.91 (1094) abBCD                        |
| 3                                                   | Nemacur® 400 EC + Kalahari 3:1                                | 0.21 (6) aA                    | 3.10 (2040) abBCD                        |
| 4                                                   | Nemacur® 400 EC +Velum® Prime 400 SC                          | 1.44 (4238) abAB               | 2.53 (1413) abBCD                        |
| 5                                                   | Nemacur® 400 EC +Velum® Prime 400 SC + Kalahari 3:1           | 0.24 (9) aA                    | 2.60 (1126) abBCD                        |
| 6                                                   | Velum® Prime 400 SC, Fluopyram @ 400 g / L, IRAC: 25; FRAC: 7 | 0.37 (119) aA                  | 2.14 (3368) abABCD                       |
| 7                                                   | Velum® Prime 400 SC + Kalahari 3:1                            | 0.30 (34) aA                   | 1.89 (1140) aABC                         |
| 8                                                   | Kalahari 3:1, organic fertiliser                              | 1.56 (450) abAB                | 3.94 (20376) bD                          |
| F-ratio:                                            |                                                               | 5.065                          | 2.653                                    |
| P-value:                                            |                                                               | 0.000                          | 0.019                                    |
| <b>Interaction: Treatments x Sampling intervals</b> |                                                               |                                |                                          |
| F-ratio:                                            |                                                               | 1.390                          |                                          |
| P-value:                                            |                                                               | 0.227                          |                                          |

<sup>1</sup>Log-transformed mean of nematode population density; <sup>2</sup>Real means of nematode population density.

### 3.3.3 Root-knot nematode damage index on potato tubers

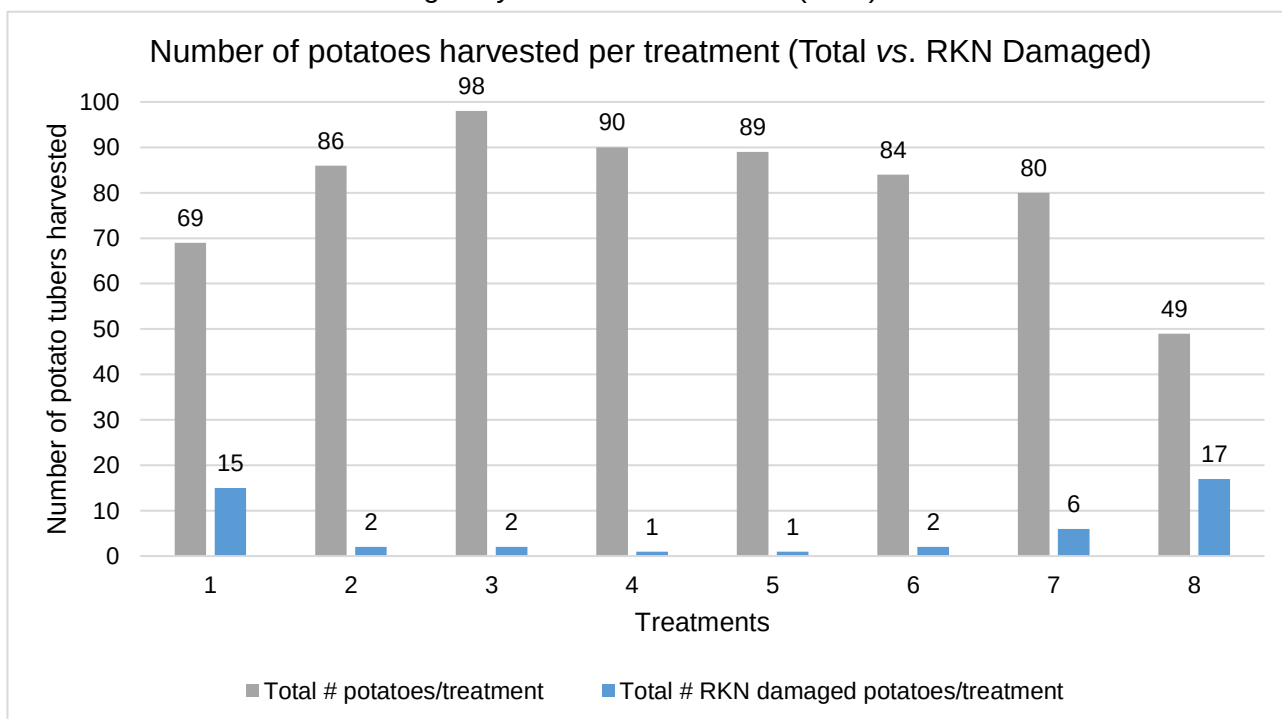
Significant differences were evident among some of the treatments at harvest (19 WAE, sampling interval 3). Treatment 8 had the highest number of RKN infected tubers and this differed significantly from most of the treatments, except treatments 1 and 7 (Table 3.14).

Figure 3.7 shows the total number of potato tubers harvested per treatment as well as the number of damaged tubers due to root-knot nematode infection (galling), while Figure 3.8 and Figure 3.9 represent the percentage infection and percentage efficacy (ABBOTT) per treatment respectively.

Table 3.14: Root-knot nematode infection (Townsend & Heuberger, 1943) data for potato tubers obtained at harvest from the study site near Tzaneen (different small letters per column indicate significant differences among treatments according to the Tukey HSD test where  $P \geq 0.05$ )

| Treatment       | Treatment name                                                | Average % tuber infection (Townsend & Heuberger) | % Efficacy (ABBOTT) |
|-----------------|---------------------------------------------------------------|--------------------------------------------------|---------------------|
| 1               | Untreated control                                             | 12,57 ab                                         | 0,00                |
| 2               | Nemacur® 400 EC, Fenamiphos @ 400 g / L, IRAC: 1B             | 1,25 a                                           | 90,05               |
| 3               | Nemacur® 400 EC + Kalahari 3:1                                | 0,38 a                                           | 96,94               |
| 4               | Nemacur® 400 EC +Velum® Prime 400 SC                          | 0,42 a                                           | 96,68               |
| 5               | Nemacur® 400 EC +Velum® Prime 400 SC + Kalahari 3:1           | 0,36 a                                           | 97,16               |
| 6               | Velum® Prime 400 SC, Fluopyram @ 400 g / L, IRAC: 25; FRAC: 7 | 1,00 a                                           | 92,04               |
| 7               | Velum® Prime 400 SC + Kalahari 3:1                            | 3,15 ab                                          | 74,93               |
| 8               | Kalahari 3:1, organic fertiliser                              | 15,52 b                                          | 0,00                |
| <b>F-ratio:</b> |                                                               | 4.131                                            |                     |
| <b>P-value:</b> |                                                               | <b>0.001</b>                                     |                     |

Figure 3.7: Number of potato tubers harvested per treatment: total number of tubers harvested vs. total number of tubers damaged by root-knot nematodes (RKN).



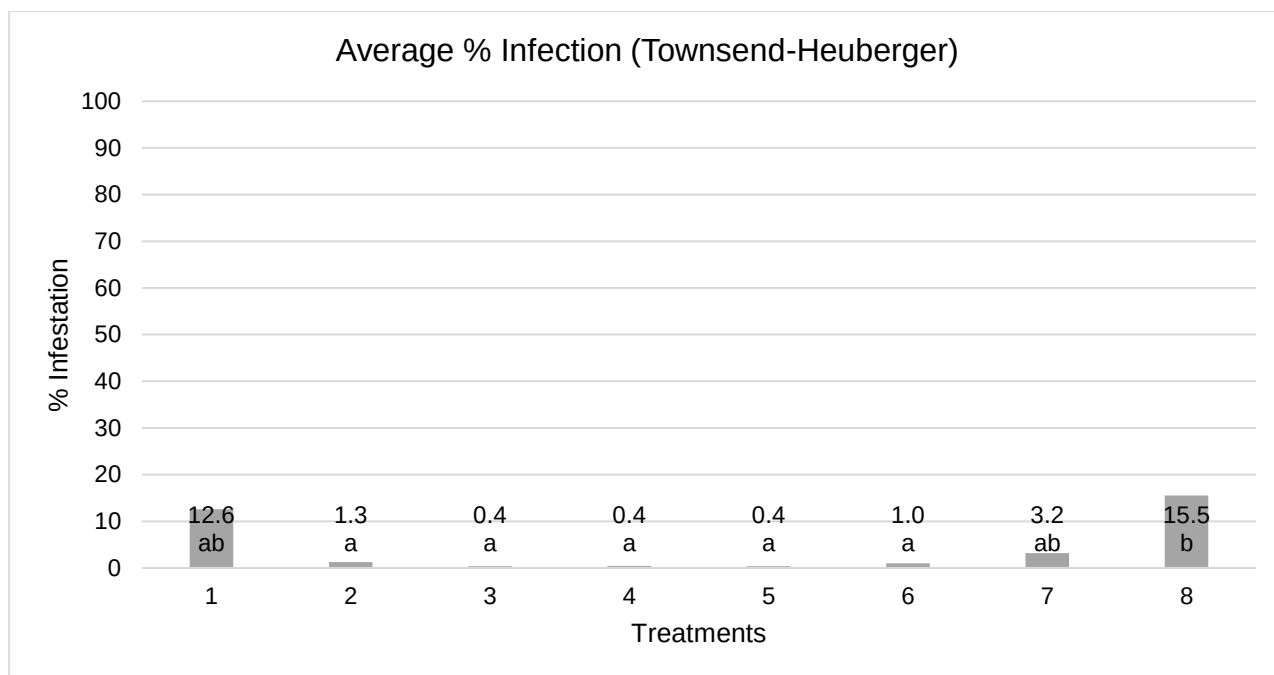


Figure 3.8: Average percentage root-knot nematode (RKN) infected potato tubers (Townsend % Heuberger, 1943) per treatment.

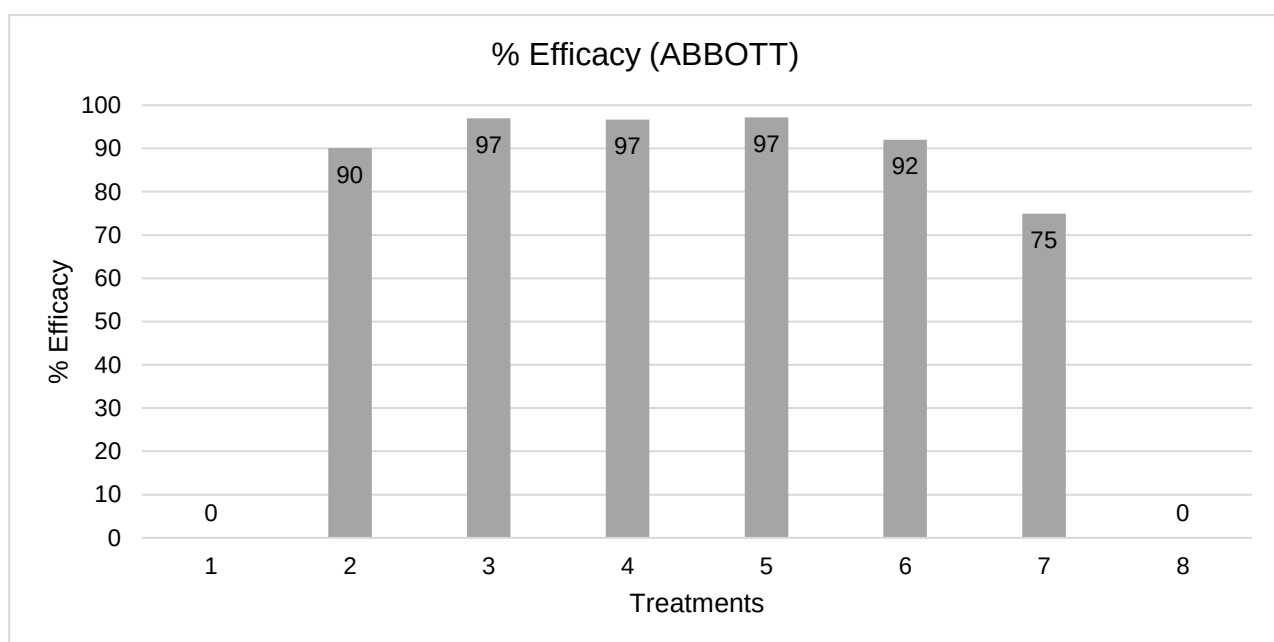


Figure 3.9: Percentage efficacy (ABBOTT) per treatment against root-knot nematode (RKN) tuber damage

### 3.4 Discussion

In this study the organic-based Kalahari 3:1 treatments, viz. neem-oil amended cow manure granule treatment, used as either a stand-alone application or combined with other nematicidal products, were not able to reduce population densities of the motile life stages of the predominant PPN genera (viz. RKN, lesion, spiral and ring nematodes). This was evident over the three sampling intervals in both the soil and root samples compared to the untreated control and for the other treatments. For PPN eggs, a similar scenario was evident except that the Kalahari 3:1 treatment in combination with Velum® Prime 400 SC (treatment 7) significantly reduced the PPN egg densities compared to the stand-alone Kalahari 3:1 treatment (treatment 8). This suggests that Velum® Prime 400 SC was the product used in this combination treatment that influenced the reproduction rate of the different PPN.

These results contradict the findings from previous studies, especially regarding the control of RKN in crops other than potato since no information on the use of a similar neem-amended manure treatment for this crop could be found for PPN in general. However, a review by Wendimu (2021) focusing on RKN management in sweet potato (*Ipomoea batatas* (L.) Lam) in Ethiopia indicated that the use of manure and neem leaf extracts often resulted in a decline in RKN populations. Results from a study by Jumaah (2015) showed that neem leaf extracts reduced galling and overall *M. incognita* populations in egg plant (*Solanum melongena* L.) in Iraq, while Kankam *et al.* (2014) showed that the use of poultry manure successfully reduced RKN populations and root galling in carrot (*Daucus carota* L.) in Ghana. Studies by Kumar and Khanna (2006) in India, Singh and Hali (2017) in Nigeria and Yadav *et al.* (2018) in India showed that the use of neem leaves, neem seed treatment and a drench or root dip, compared to treatments that were not treated with neem-based products, reduced RKN reproduction in tomato (cv. 'Glamour') by 37.4%, 67.5 % and 23.5% respectively.

During this study, the few tubers that were obtained from the trial site showed that all the treatments containing one or both chemical nematicides had more tubers present, while the treatment consisting of only the organic product Kalahari 3:1 had the lowest number of tubers compared to all the treatments, including the untreated control (Table 3.14 and Figures 3.7 and 3.9). The findings from this study seemed to contradict those by other authors and are difficult to explain at this stage, but as noted later in this section, the severe drought experienced in the trial site area in the Deerpark (Tzaneen) area is suggested to have had a considerable effect on nematode data and number of tubers harvested. It is known that soil amendments generally improve soil structure and quality, resulting in reduced stress on the plants, while some amendments can also exhibit nematicidal properties (Khalil, 2013; Onkendi *et al.*, 2014). Shayaa and Hussein (2019) in Iraq compared the use of neem leaf extract, cow manure and different

combinations of neem extract with 5% cow manure on the growth and yield of two potato cultivars viz. Burren and Riviera. Their findings indicated that combinations of cow manure with neem leaf extract improved the overall potato growth and yield significantly compared to the untreated control and chemical fertiliser applications (Shayaa & Hussein, 2019). The combination of cow manure with neem leaf extracts resulted in healthier crops that can naturally resist or outgrow the potential of PPN to attack their root systems (Dutta *et al.*, 2019). Hajji and Horrigue-Raouani (2012) also observed better potato (cv. Spunta) emergence and development in the treatments containing manure and compost in Tunisia, although RKN densities were not reduced by these treatments.

A previous study investigating the nematicidal potential of neem leaf extracts and poultry manure on sweet potato in Ghana indicated that the addition of neem leaf extracts had also been successful in reducing especially LN populations significantly compared to untreated plots and plots treated with poultry manure. The nematicidal effect of the manure treatment showed variable results between different cultivars used (Osei *et al.*, 2017). This significant reduction in LN population numbers due to the addition of neem leaves, or neem leaf extracts, is supported by several studies on various crops: Radish (*Raphanus sativus* L. 'Early Scarlet Globe'), cucumber (*Cucumis sativus* L. 'Marketmore 76') and tomato in Canada (Abbasi *et al.*, 2005), cucumber in Ghana (Agyarko & Asante, 2005), black pepper (*Piper nigrum* L.) in Vietnam (Hieu *et al.*, 2014) and crossandra (*Crossandra undulaefolia* L.) in India (Jothi *et al.*, 2004). A study by Khan *et al.* (2015) on peach (*Prunus persica* (L.) Batsch) seedlings in Pakistan found that, although the neem powder reduced LN population numbers, the chemical nematicide standard carbofuran provided superior control. Ali *et al.* (2021) found that the use of different animal manures (chicken, sheep and cow) each increased plant growth in cucumber in Egypt significantly while also reducing the population densities of various PPN, including LN. A study conducted by Cole *et al.* (2020) to control LN in potato in Michigan, United States of America (USA) had similar findings using poultry manure. A recent study by Izuogu and Usman (2019) in Nigeria showed that making a compost tea from either poultry or cow manure significantly reduced LN population densities in maize (*Zea mays* L.). The findings from this Deerpark study correlates to a lesser degree with the findings from these authors, in that all the treatments, including the neem-amended cow manure treatments, reduced the population densities of LN, even though only NemaCur 400 EC stand alone and the combination of all three products together showed statistically significant differences from the untreated control.

Previous research focussing on the use of neem amendments and animal manure on other nematodes, such as spiral (Hoplolaimidae) and ring (Criconematidae) nematodes showed that the use of neem leaves and especially poultry and cow manure reduced these populations

significantly (Agyarko & Asante, 2005; Ali *et al.*, 2021; Khan *et al.*, 2015; Osei *et al.*, 2017), although this was not observed in this study. Although the population densities showed a slight decline over time, this could not be attributed directly to the application of the neem-amended cow manure granules. It must be kept in consideration that the nematode numbers of these nematodes were generally low, compared to what is known and experienced in potato fields in South Africa (Jones *et al.*, 2017), throughout the trial period, making it more difficult to demonstrate significant differences.

The lack of effect by the neem-amended cow manure granule on the number of PPN eggs produced contradicts those by Hayat *et al.* (2012) in Pakistan who found that potato plants grown in soil amended with ground neem seeds produced significantly less RKN eggs than the untreated control and other soil amendments. Controversy in results from the Deerpark study and the Pakistan study is difficult to explain but severe drought conditions experienced in the trial site are most probably the greatest contributor towards the inferior performance of the tried and tested synthetically derived nematicides. Such nematicides are known to be effective in reducing PPN in local potato fields (Sesweni, 2016), with the same explanation being proposed for the Kalahari 3:1 product performing non-optimal in this study.

Results from this study, however, strongly correlate with the review of different RKN management strategies by Collange *et al.* (2011) in different vegetable crops indicating that the application of organic amendments, including neem extracts in larger field studies, have shown variable or inconclusive results with substantial variation existing between the studies referred to. Except for severe drought conditions, another possibility is related to the product itself in that the concentration of neem extract in the cow manure granules used was too low and therefore not sufficient to effectively reduce RKN and other PPN numbers. It might thus be interesting to investigate a different formulation with a higher concentration of the neem extract. Another possible explanation why the Kalahari 3:1 treatment was not effective in the present study could be, as in previous research, neem leaves, instead of a leaf extract and raw (ungranulated) manure, provided better and more consistent results (Abbasi *et al.*, 2005; Agyarko & Asante, 2005; Hieu *et al.*, 2014; Jothi *et al.*, 2004; Khan *et al.*, 2015; Osei *et al.*, 2017; Shayaa & Hussein, 2019). It is important to then also again accentuate that the drought might have had a considerable effect on results obtained in this trial since a lack of sufficient soil moisture puts considerable strain on plant growth, plant vigour, nematode survival and population development. A severe drought was experienced in the area from the summer of 2014/2015 up until early in the 2019/2020 rainy season with rainfall averaging between 50 and 75% of the long-term figures. The average rainfall on the farm where the trial was conducted before the drought was approximately 1 100 mm per season (personal records of Pieter Buys, farm owner, dating back to the 2008/2009

season). The rainfall records from 2008 are shown in Figure 3.5 and indicate the rainfall per month, per season, of Nyalani Estates, the farm on which the trial was conducted. Plant-parasitic nematodes are aquatic animals that require sufficient levels of soil water to survive and reproduce (Perry, 1996), meaning their survival and motility was likely limited to the areas in the soil that received drip irrigation, which could not be optimally applied due to the severe drought and low levels of water in the cement storage dam on the farm situated about 900m away from the trial site. Water for agriculture was restricted with household use receiving priority from the start of 2019 until the start of 2021.

In essence, the use of drip irrigation would concentrate the PPN in the immediate crop root zone. However, with restricted irrigation being allowed for agricultural purposes combined with reduced rainfall, the adverse effect on the efficacy of the products tested was expected, as the performance of the products was not optimal in this trial. It is known that drought impact adversely on the efficacy of nematicides as they must be transported and dissolved in this aqueous medium to ensure optimal efficacy (Jones *et al.*, 2017). Riekert (1996) explicitly mentioned that drought periods impacted adversely on nematicide performance in maize trials. The water supplied by irrigation at the Deerpark trial site during this study was firstly not sufficient to bring the soil water back to capacity and could have played a role in the spread of the products around the crop root zone. This could also have played a role in the ability of the plants to take up the fertiliser component of the Kalahari 3:1 treatments. The reduced amount of rainfall between 2014 - 2020 is clearly shown in Figure 3.5, suggesting that one of the reasons for the poor performance in this trial was due to lack of sufficient moisture. The temperature records for the Tzaneen area, shown in Figure 3.6, only show two abnormal spikes in the average maximum temperatures (approximately March 2014 and again between December 2015 – January 2016). This suggests that temperature did not play as big a role in the results of this trial as the amount of rainfall experienced.

Moreover, except for the proposed negative effect of the drought and potential insufficient content of the neem and manure ingredients in Kalahari 3:1 (to optimally act as a nematicide), the RKN *M. enterolobii*, occurring at the site in combination with *M. javanica*, is known to be more virulent than other RKN species (Castagnone-Sereno, 2012), which may have been an additional contributing factor to the poor performance of the products in this trial, while some populations of *M. javanica* are also known to be quite virulent (Ornat *et al.*, 2007), which could also have influenced the results obtained.

### 3.5 Conclusion

From the results and discussions, the treatment consistently showing the best effect against the different PPN extracted from both the soil and roots was treatment 4 (Nemacur 400 EC @ 25L/ha + Velum Prime 400 SC @ 2.5L/ha), although it was not significant. Both products are registered products known to be effective in reducing PPN population numbers (Dyrdahl-Young *et al.*, 2020; Pofu & Mashela, 2021; Seshweni, 2016). In cases where populations declined, even in the untreated control, the decline was most likely due to environmental factors such as heat and drought and the subsequent decline in a stable food source.

Contrary to expectations, the organic manure amended with neem oil when used alone seemed to have very little effect on PPN populations and in some cases seemed to increase the rate at which population numbers increased. When combined with a 'chemical' nematicide, however, several treatments resulted in a slightly improved performance against some of the PPN populations in the soil. More research is needed investigating higher concentrations of neem oil in the Kalahari 3:1 granule as well as using other forms of neem (leaves, seedcake, ground seeds, etc.) in combination with an organic, granulated cow manure to determine if these strategies would yield better management of PPN. These studies should also be conducted in more favourable growing seasons to reduce the possible effect that unfavourable conditions could have on the overall potato crop. Furthermore, the occurrence of virulent RKN populations must always be kept in mind in such research as they also have a negative impact on the performance of management strategies that are known to be effective.

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## **Chapter 4: Effects of a neem-amended product and a successive grass fallow on beneficial soil nematode population densities present in a potato field**

### **4.1 Introduction**

Beneficial soil nematodes constitute a substantial part of the total soil nematode community and are essential for healthy, productive soils, as discussed in Chapter 1, par. 1.3.4, Table 1.3 and Figure 1.2 (Nemaplex, 2021; Yeates *et al.*, 1993). Beneficial soil nematodes regulate the populations of other soil micro-organisms, mineralise nutrients into plant-available forms, provide a food source for other soil organisms and consume disease-causing organisms (Ingham, 2000). They have diverse feeding strategies that are used to classify them into different trophic groups, viz. fungivores, bacterivores, omnivores and predators. They are also essential for a range of activities and functions in the soil food web, which include nitrogen fixing, nutrient cycling and decomposition of organic matter into easily accessible nutrients for plants/crops (Buchan *et al.*, 2013; Ferris *et al.*, 2004; Stenberg, 1999). The characteristics of beneficial soil nematodes can be and are used to give an indication of the health/quality of soil, thus making them good bio-indicators, especially in soil used for agricultural practices (Ferris *et al.*, 2004; Ingham *et al.*, 1985; McSorley, 2019).

A bio-indicator is defined as an aggregate term referring to all sources of biotic and abiotic reactions to ecological changes according to Parmar *et al.* (2016). Thus, bio-indicators can be used to assess and monitor environmental health and to detect positive or negative environmental changes. Beneficial soil nematodes react swiftly to environmental changes, making them good bio-indicators for soil health (Maurya *et al.*, 2020; Parmar *et al.*, 2016). The Soil Science Society of America Ad Hoc Committee on soil quality (S-581) defined healthy soil as “the continued capacity of soil to function as a vital living system, within the ecosystem and land-use boundaries, to sustain biological productivity, promote the quality of air and water environments and maintain plant, animal and human health” (Doran *et al.*, 1996; Doran & Safley, 1997; Van Bruggen & Semenov, 2000). Soil health thus refers to the physical, chemical and biological characteristics of the soil and how these affect the ability of the soil to facilitate plant growth and other environmental functions (Cardoso *et al.*, 2013; Karlen *et al.*, 1997; Munoz-Rojas, 2018).

Soil nematode communities tie into the microbial biomass present in the soil and the process of using these communities as bioindicators is elaborated in detail in Chapter 1, par. 1.3.4, which refers to the use of trophic groups, the division of beneficial nematodes into coloniser-persister (c-p) classes and the designation of a Maturity Index (MI) to each coloniser-persister (c-p) class to determine the stability or maturity of a selected soil ecosystem (Bongers & Bongers, 1998; Ferris *et al.*, 2004; Nemaplex, 2021; Sieriebriennikov *et al.*, 2014).

Various activities applied to soil have different effects on the soil beneficial nematode populations and other microbes present. Studying the effect of these activities on beneficial soil nematodes could assist in developing and implementing more sustainable agricultural practices (Kapp, 2013; Moura & Franzener, 2017; Sofo *et al.*, 2021). Such activities and conditions may also determine the establishment or recovery rate of these beneficial nematodes. Agricultural practices, such as tillage and monocropping systems have a significant effect on various microbial processes in the soil (Sofo *et al.*, 2021). Studies by Pfozner and Schuler (1997) on potato (*Solanum tuberosum* L.) in Germany and Forge *et al.* (2003) on apple (*Malus domestica* Borkh) in Canada, showed that the application of organic compounds favoured biological activities and increased the abundance of beneficial soil nematodes, thus increasing the release of plant available nutrients. Goulart and Ferraz (2003) showed that clearing natural areas in the Cerrado area in Brazil, to cultivate guava (*Psidium guajava* L.) and maize (*Zea mays* L.) reduced the abundance of predatory and omnivore nematodes and reduced the overall trophic diversity of the area. This reduction in beneficial nematodes, especially nematodes from the family Dorylaimidae, was also observed by Torres *et al.* (2006) in melon (*Cucumis melo* L.) plants cultivated in Brazil. A short fallow period was not sufficient for the beneficial soil nematodes to recover (Torres *et al.*, 2006). The family Dorylaimidae is overly sensitive to agricultural practices, making it especially useful as a bioindicator of environmental disruptions (Gomes *et al.*, 2003). Conventional tillage distributes any crop residues throughout the ploughed layer, reducing the stability and availability of the soil organic matter (SOM). Conventional agricultural practices also cause great soil disturbances, increase the amount of CO<sub>2</sub> which is released and also increase the potential of soil erosion when compared to zero tillage fields (Kushwaha *et al.*, 2001; Sofo *et al.*, 2021). Conventional agricultural practices seem to reduce the stability of beneficial nematode populations while increasing the populations of plant-parasitic nematodes (PPN) in the crop rhizosphere (Briar *et al.*, 2007).

Plant or crop diversity also plays a key role in the diversity and abundance of different nematode trophic groups (Gebremikael *et al.*, 2016; Puissant *et al.*, 2021). Puissant *et al.* (2021) concluded that increasing plant diversity will significantly affect the taxonomic richness and MI of a soil. This means that cover cropping systems will improve the diversity and abundance of the beneficial soil nematode populations in the soil compared to a bare fallow period (Kim *et al.*, 2020).

The aims of this study were to determine: i) what effect a neem oil-amended cow manure granule, marketed as Kalahari 3:1, as a stand-alone but also in combination with registered nematicides could have on beneficial soil nematodes under typical South African field conditions in a potato planting near Tzaneen (Limpopo province), ii) how these effects compare to that of registered

nematicides on beneficial soil nematodes and iii) what the effect of a successive 27 month grass fallow is on the beneficial soil nematode community composition compared to that at pre-plant.

## 4.2 Materials and methods

### 4.2.1 Site and design

The same site, trial layout and treatments as discussed in Chapter 3, par 3.2.1 – 3.2.2 were used for the collection of beneficial soil nematode data.

### 4.2.2 Nematode sampling and analyses: root and soil

The dates of soil and root samplings are shown in Table 4.1. The methods of sampling, extraction and counting were discussed in Chapter 3, par. 3.2.3-3.2.5. An additional sample timing was added for this specific part of the study to determine the effect of the grass fallow period on the beneficial nematode community composition. In this study, the field was allowed to recover naturally and the predominant vegetation that grew during the fallow period, commencing after the evaluation of the Kalahari 3:1 treatments, was White Buffalo grass (*Urochloa mosambicensis* (Hack.) Dandy. (Family: Poaceae)) (Malan, 2018; Mashau, 2010), a grass species that naturally occurs in agricultural areas.

Table 4.1: Sampling intervals of soil and roots for nematode analyses from the trial site situated on Nyalani Estates, Deerpark near Tzaneen.

| Sample timing | Dates           | Soil | Roots |
|---------------|-----------------|------|-------|
| Pre-plant     | 5 October 2018  | Yes  | No    |
| 10 WAE        | 18 January 2018 | Yes  | Yes   |
| Harvest       | 25 March 2019   | Yes  | Yes   |
| After fallow  | 1 June 2021     | Yes  | Yes   |

### 4.2.3 Statistical Analysis

For statistical analysis, the beneficial soil nematodes extracted at the first three sample intervals were grouped into trophic levels and transformed using  $\log_{10}(x+1)$ , which were then subjected to One-way ANOVA (Statistica 14.0.0) to compare the effect of the different treatments, per sample date. The data was also subjected to Repeated Measures ANOVA, using Statistica 14.0.0 ([www.tibco.com](http://www.tibco.com)) to investigate the effect of treatment over the four sampling intervals on the beneficial nematode community. The level of significance ( $P < 0.05$ ) was determined using the Tukey HSD test, where  $P = 0.05$ .

Principal Component Analysis (PCA) using the ADE-4 program, 2001 Version (Thioulouse *et al.*, 1997) was used to visualise whether any correlations existed between the different soil factors and beneficial nematode communities present during the respective sampling intervals.

The Nematode Indicator Joint Analysis (NINJA) tool, developed by Sieriebriennikov *et al.* (2014) to assist in studying soil ecosystems, was also used to determine what role nematode bioindicators played in the specific trial site in terms of soil health. Data sets, including nematode genera/families of beneficial nematodes determined at pre-plant and 27 months later, were imported in NINJA. The various indices used to study nematode populations in an ecosystem were discussed in Chapter 1, par 1.3.4. The tool utilises the different indices assigned to nematode families/genera to determine soil health.

### **4.3. Results**

#### **4.3.1 Beneficial nematode data from 250 ml soil**

Beneficial nematodes were grouped into trophic levels for statistical analysis at the first 3 sampling intervals. The diversity and population densities recorded in the soil were for fungivores, bacterivores and omnivores. The total counts for beneficial nematodes were also subjected to One-way and Repeated Measures ANOVA.

##### **4.3.1.1 Total beneficial nematode counts from soil**

No significant differences were evident among the treatments for sampling intervals 1 and 3 (Table 4.2). Significant differences were evident among the treatments for sampling interval 2, where treatments 1 and 8 differed significantly from treatments 4 and 5. A significant interaction existed for treatments x sampling times, indicating that total beneficial soil nematode population densities differed between some treatments and sampling times. Population densities of these beneficial soil nematodes increased over time for all treatments except for treatment 4, with the highest abundance evident during sampling interval 3.

Population densities for total beneficial soil nematodes in 250 ml soil samples were relatively high, before the commencement of treatment applications and planting activities (Pre-plant sampling interval), ranging from 694 to 2213/250 ml soil (Table 4.2). At 10 weeks after crop emergence (WAE; Sampling interval 2), only plots treated with treatments 4 or 5 showed a decline in population densities, which differed significantly from the nematode numbers in treatments 1 and 8. By the harvest sampling date, (19 WAE; Sampling interval 3) all the treatments showed an increase in beneficial soil nematode counts which did not differ significantly from each other. Only treatment 8 increased significantly over time.

Table 4.2: Totals of beneficial nematode population densities in 250 ml soil. (Significance level for Repeated measures ANOVA in capital letters; different letters indicate significant differences across rows for sampling intervals; different small letters per column indicate significant differences among treatments per individual sampling interval).

| Treatment                                           | Treatment names                                                  | Sampling interval 1:<br>Pre-plant         | Sampling interval 2:<br>10 WAE | Sampling interval 3:<br>19 WAE (Harvest) |
|-----------------------------------------------------|------------------------------------------------------------------|-------------------------------------------|--------------------------------|------------------------------------------|
| 1                                                   | Untreated control                                                | 2.91 <sup>1</sup> (950) <sup>2</sup> aABC | 3.43 (3325) bC                 | 3.4 (3038) aC                            |
| 2                                                   | Nemacur® 400 EC,<br>Fenamiphos @ 400 g / L,<br>IRAC: 1B          | 2.94 (1050) aABC                          | 3.13 (1581) abBC               | 3.39 (2863) aC                           |
| 3                                                   | Nemacur® 400 EC + Kalahari 3:1                                   | 2.46 (694) aAB                            | 3.26 (2419) abABC              | 3.26 (2238) aBC                          |
| 4                                                   | Nemacur® 400 EC +Velum® Prime 400 SC                             | 3.2 (2213) aBC                            | 2.91 (950) aABC                | 3.17 (2000) aABC                         |
| 5                                                   | Nemacur® 400 EC +Velum® Prime 400 SC + Kalahari 3:1              | 2.92 (1131) aABC                          | 2.83 (731) aABC                | 3.12 (1663) aABC                         |
| 6                                                   | Velum® Prime 400 SC,<br>Fluopyram @ 400 g / L, IRAC: 25; FRAC: 7 | 2.93 (988) aABC                           | 3.26 (2300) abBC               | 3.3 (3306) aBC                           |
| 7                                                   | Velum® Prime 400 SC + Kalahari 3:1                               | 3.18 (1738) aABC                          | 3.2 (2006) abBC                | 3.38 (2638) aC                           |
| 8                                                   | Kalahari 3:1, organic fertiliser                                 | 2.34 (1013) aA                            | 3.44 (3256) bC                 | 3.51 (3788) aC                           |
| <b>F-ratio:</b>                                     |                                                                  | 1.597                                     | 4.526                          | 1.402                                    |
| <b>P-value:</b>                                     |                                                                  | 0.155                                     | <b>0.000</b>                   | 0.223                                    |
| <b>Interaction: Treatments x Sampling intervals</b> |                                                                  |                                           |                                |                                          |
| <b>F-ratio:</b>                                     |                                                                  | 2.47                                      |                                |                                          |
| <b>P-value:</b>                                     |                                                                  | <b>0.004</b>                              |                                |                                          |

<sup>1</sup>Log-transformed mean of nematode population density; <sup>2</sup>Real means of nematode population density.

#### 4.3.1.2 Bacterivore nematode counts from soil

Statistically significant differences were observed between treatments 3 and 7 (with significantly higher bacterivore abundance) at pre-plant, while treatment 8 differed significantly from all the other treatments with the highest mean of bacterivores observed at WAE. No significant differences were observed between treatments at the harvest sampling interval (Table 4.3). Over the three sampling intervals, significant interaction was evident for treatments 1 and 3. The abundance of bacterivores for treatments 1 and 3 at pre-plant were significantly lower than that of sampling intervals 2 and 3.

Population densities of bacterivores in 250 ml soil were high at pre-plant (sampling interval 1), ranging from 203 to 1669/250 ml soil (Table 4.3). Population densities generally increased at 10 WAE (sampling interval 2), except for treatments 4 and 5, ranging from 650 to 2563/250 ml soil. Population densities increased at harvest (sampling interval 3) as well, ranging from 1469 to 3494/250 ml soil.

Table 4.3: Bacterivore nematode population densities in 250 ml soil. (Significance level for Repeated measures ANOVA in capital letters; different letters indicate significant differences across rows for sampling intervals; different small letters per column indicate significant differences among treatments per individual sampling interval).

| Treatment                                           | Treatment name                                                   | Sampling interval 1:<br>Pre-plant         | Sampling interval 2:<br>10 WAE | Sampling interval 3:<br>19 WAE (Harvest) |
|-----------------------------------------------------|------------------------------------------------------------------|-------------------------------------------|--------------------------------|------------------------------------------|
| 1                                                   | Untreated control                                                | 2.13 <sup>1</sup> (641) <sup>2</sup> abAB | 3.23 (2239) aC                 | 3.23 (2025)aC                            |
| 2                                                   | Nemacur® 400 EC,<br>Fenamiphos @ 400 g / L,<br>IRAC: 1B          | 2.81 (880) abBC                           | 3.09 (1456) aBC                | 3.32 (2556) aC                           |
| 3                                                   | Nemacur® 400 EC + Kalahari 3:1                                   | 1.55 (203) aA                             | 3.21 (2213) aC                 | 3.23 (2100) aC                           |
| 4                                                   | Nemacur® 400 EC +Velum® Prime 400 SC                             | 2.90 (1669) abBC                          | 2.83 (744) aBC                 | 3.04 (1638) aBC                          |
| 5                                                   | Nemacur® 400 EC +Velum® Prime 400 SC + Kalahari 3:1              | 2.40 (756) abABC                          | 2.79 (650) aBC                 | 3.06 (1469) aBC                          |
| 6                                                   | Velum® Prime 400 SC,<br>Fluopyram @ 400 g / L, IRAC: 25; FRAC: 7 | 2.73 (615) abBC                           | 3.17 (2050) aBC                | 3.16 (2763) aBC                          |
| 7                                                   | Velum® Prime 400 SC + Kalahari 3:1                               | 3.05 (1469) bBC                           | 2.96 (1188) aBC                | 3.23 (1906) aC                           |
| 8                                                   | Kalahari 3:1, organic fertiliser                                 | 2.64 ( 938) abBC                          | 3.29 (2563) bC                 | 3.45 (3494) aC                           |
| <b>F-ratio:</b>                                     |                                                                  | 2.356                                     | 2.783                          | 1.286                                    |
| <b>P-value:</b>                                     |                                                                  | <b>0.035</b>                              | <b>0.015</b>                   | 0.274                                    |
| <b>Interaction: Treatments x Sampling intervals</b> |                                                                  |                                           |                                |                                          |
| <b>F-ratio:</b>                                     |                                                                  | 2.60                                      |                                |                                          |
| <b>P-value:</b>                                     |                                                                  | <b>0.003</b>                              |                                |                                          |

<sup>1</sup>Log-transformed mean of nematode population density; <sup>2</sup>Real means of nematode population density.

#### 4.3.1.3 Fungivore nematode counts from soil

Significant differences were observed at 10 WAE only, which was evident between treatments 2 and 3 differing significantly from treatment 1 (Table 4.4). There were no statistically significant differences between treatments at the pre-plant and harvest sampling intervals, nor were there any significant interactions between treatments x sampling intervals. This indicates that, overall, fungivore population densities were similar for most treatments and sampling times, although they increased over time for treatments 1, 2, 4, 6 and 7 but not for treatments 3, 5 and 8.

Fungivore population densities in 250 ml soil were moderately low, ranging from 100 to 488/250 ml soil at pre-plant (sampling interval 1) (Table 4.4). Population densities increased slightly at 10 WAE (sampling interval 2), ranging from 81 to 1004/250 ml soil. Population densities at harvest (sampling interval 3) generally remained similar to that at 10 WAE, ranging from 106 to 950/250 ml soil.

Table 4.4: Fungivore nematode population densities in 250 ml soil. (Significance level for Repeated measures ANOVA in capital letters; different letters indicate significant differences across rows for sampling intervals; different small letters per column indicate significant differences among treatments per individual sampling interval).

| Treatment                                               | Treatment name                                                   | Sampling interval 1:<br>Pre-plant       | Sampling interval 2:<br>10 WAE | Sampling interval 3:<br>19 WAE (Harvest) |
|---------------------------------------------------------|------------------------------------------------------------------|-----------------------------------------|--------------------------------|------------------------------------------|
| 1                                                       | Untreated control                                                | 2.11 <sup>1</sup> (250) <sup>2</sup> aA | 2.89 (1004) bA                 | 2.26 (950) aA                            |
| 2                                                       | Nemacur® 400 EC,<br>Fenamiphos @ 400 g / L,<br>IRAC: 1B          | 1.91 (176) aA                           | 1.34 (113) aA                  | 1.57 (219) aA                            |
| 3                                                       | Nemacur® 400 EC + Kalahari 3:1                                   | 2.31 (488) aA                           | 1.36 (156) aA                  | 1.54 (106) aA                            |
| 4                                                       | Nemacur® 400 EC +Velum® Prime 400 SC                             | 1.68 (194) aA                           | 1.47 (206) abA                 | 2.46 (350) aA                            |
| 5                                                       | Nemacur® 400 EC +Velum® Prime 400 SC + Kalahari 3:1              | 1.85 (269) aA                           | 1.46 (81) abA                  | 1.72 (188) aA                            |
| 6                                                       | Velum® Prime 400 SC,<br>Fluopyram @ 400 g / L, IRAC: 25; FRAC: 7 | 1.46 (210) aA                           | 1.97 (194) abA                 | 1.92 (456) aA                            |
| 7                                                       | Velum® Prime 400 SC + Kalahari 3:1                               | 1.32 (100) aA                           | 2.68 (813) abA                 | 1.87 (688) aA                            |
| 8                                                       | Kalahari 3:1, organic fertiliser                                 | 1.85 (256) aA                           | 2.5 (644) abA                  | 1.34 (256) aA                            |
| <b>F-ratio:</b>                                         |                                                                  | 0.719                                   | 3.681                          | 0.848                                    |
| <b>P-value:</b>                                         |                                                                  | 0.656                                   | <b>0.002</b>                   | 0.552                                    |
| <b>Interaction:<br/>Treatments x Sampling intervals</b> |                                                                  |                                         |                                |                                          |
| <b>F-ratio:</b>                                         |                                                                  |                                         | 1.615                          |                                          |
| <b>P-value:</b>                                         |                                                                  |                                         | 0.086                          |                                          |

<sup>1</sup>Log-transformed mean of nematode population density; <sup>2</sup>Real means of nematode population density.

#### 4.3.1.4 Omnivore nematode counts from soil

Statistically significant differences were only observed at pre-plant, with treatments 2 and 3 differing significantly from treatment 7 but not from the others (Table 4.5). No statistically significant differences were observed for omnivore nematodes between treatments at 10 WAE and harvest sampling intervals. Some significant interactions did exist between treatments x sampling intervals. Treatments 2 and 3 at pre-plant and treatments 4 and 5 at 10 WAE differed significantly from treatment 7 at pre-plant. Population densities varied over time.

Omnivore population densities in 250 ml soil varied, ranging from zero to 263/250 ml soil at pre-plant (sampling interval 1) (Table 4.5). Population densities declined at 10 WAE (sampling interval 2), ranging from zero to 81/250 ml soil. A slight increase was observed in population densities at harvest (sampling interval 3), ranging from six to 88/250 ml soil.

Table 4.5: Omnivore nematode population densities in 250 ml soil. (Significance level for Repeated measures ANOVA in capital letters; different letters indicate significant differences across rows for sampling intervals; different small letters per column indicate significant differences among treatments per individual sampling interval).

| Treatment                                               | Treatment name                                                   | Sampling interval 1:<br>Pre-plant        | Sampling interval 2:<br>10 WAE | Sampling interval 3:<br>19 WAE (Harvest) |
|---------------------------------------------------------|------------------------------------------------------------------|------------------------------------------|--------------------------------|------------------------------------------|
| 1                                                       | Untreated control                                                | 0.97 <sup>1</sup> (59) <sup>2</sup> abAB | 0.61 (81) aAB                  | 0.83 (63) aAB                            |
| 2                                                       | Nemacur® 400 EC,<br>Fenamiphos @ 400 g / L,<br>IRAC: 1B          | 0 (0.0) aA                               | 0.43 (13) aAB                  | 1.27 (88) aAB                            |
| 3                                                       | Nemacur® 400 EC + Kalahari 3:1                                   | 0 (0.0) aA                               | 0.57 (50) aAB                  | 0.3 (31) aAB                             |
| 4                                                       | Nemacur® 400 EC +Velum® Prime 400 SC                             | 1.34 (263) abAB                          | 0 (0.0) aA                     | 0.43 (13) aAB                            |
| 5                                                       | Nemacur® 400 EC +Velum® Prime 400 SC + Kalahari 3:1              | 1.01 (63) abAB                           | 0 (0.0) aA                     | 0.21 (6) aAB                             |
| 6                                                       | Velum® Prime 400 SC,<br>Fluopyram @ 400 g / L, IRAC: 25; FRAC: 7 | 1.44 (169) abAB                          | 0.33 (56) aAB                  | 1.12 (88) aAB                            |
| 7                                                       | Velum® Prime 400 SC + Kalahari 3:1                               | 1.8 (213) bB                             | 0.21 (6) aAB                   | 0.52 (44) aAB                            |
| 8                                                       | Kalahari 3:1, organic fertiliser                                 | 1.41 (119) abAB                          | 0.53 (50) aAB                  | 0.91 (38) aAB                            |
| <b>F-ratio:</b>                                         |                                                                  | 3.322                                    | 0.72                           | 1.279                                    |
| <b>P-value:</b>                                         |                                                                  | <b>0.005</b>                             | 0.655                          | 0.278                                    |
| <b>Interaction:<br/>Treatments x Sampling intervals</b> |                                                                  |                                          |                                |                                          |
| <b>F-ratio:</b>                                         |                                                                  | 1.97                                     |                                |                                          |
| <b>P-value:</b>                                         |                                                                  | <b>0.026</b>                             |                                |                                          |

<sup>1</sup>Log-transformed mean of nematode population density; <sup>2</sup>Real means of nematode population density.

#### 4.3.2 Principal component analysis

The principal component analysis (PCA) of nematodes for the pre-plant sampling interval showed that the populations found in the soil samples of the different treatments were remarkably similar in diversity and abundance (Figure 4.1 a & b).

At the 10 WAE sampling date, the untreated control (treatment 1) and treatment 8 seemed to correlate with higher levels of nematodes, which included fungivore and bacterivore nematodes, PPN such as lesion (LN, *Pratylenchus*, Filipjev, 1936), spiral (Hoplolaimidae, Filipjev, 1934) and kidney shaped (*Rotylenchulus*, Lindford & Oliveira, 1940) nematodes (Figure 4.2 a & b). Most of the other treatments were clustered to the left of the Y-axis, indicating small numbers of nematodes from both soil and root samples.

At harvest (sampling interval 3), treatments 1 and 8 were positioned away from the other treatments, indicating higher numbers of second-stage juveniles RKN (J2) and eggs, ring

(Criconematidae, Taylor, 1936), lesion, spiral, bacterivore and fungivore nematodes (Figure 4.3 a & b). The other treatments were clustered together and had overall lower nematode numbers.

A coinertia analysis between the chemo-physical properties of the soil and the log transformed nematode data from the soil and roots at 10 WAE (Figure 4.4) indicated that LN, spiral, ring and fungivores were associated with higher clay percentages and Al (mg/kg) levels. Root-knot nematodes (females) and stubby root nematodes (from both soil and root samples) were linked to higher pH levels, Ca/Mg, P (mg/kg) and K (mg/kg).

Coinertia analysis between the chemo-physical properties of the soil and the log transformed nematode data from the soil and roots at harvest (Figure 4.5) indicated that RKN (J2, eggs and females) and sheath nematodes were positively associated with clay percentage. Spiral nematodes in the soil were positively associated with higher levels Al (mg/kg) while stubby root and stunt nematodes were strongly associated with higher P, pH, Ca (mg/kg) and Mg (mg/kg) levels.

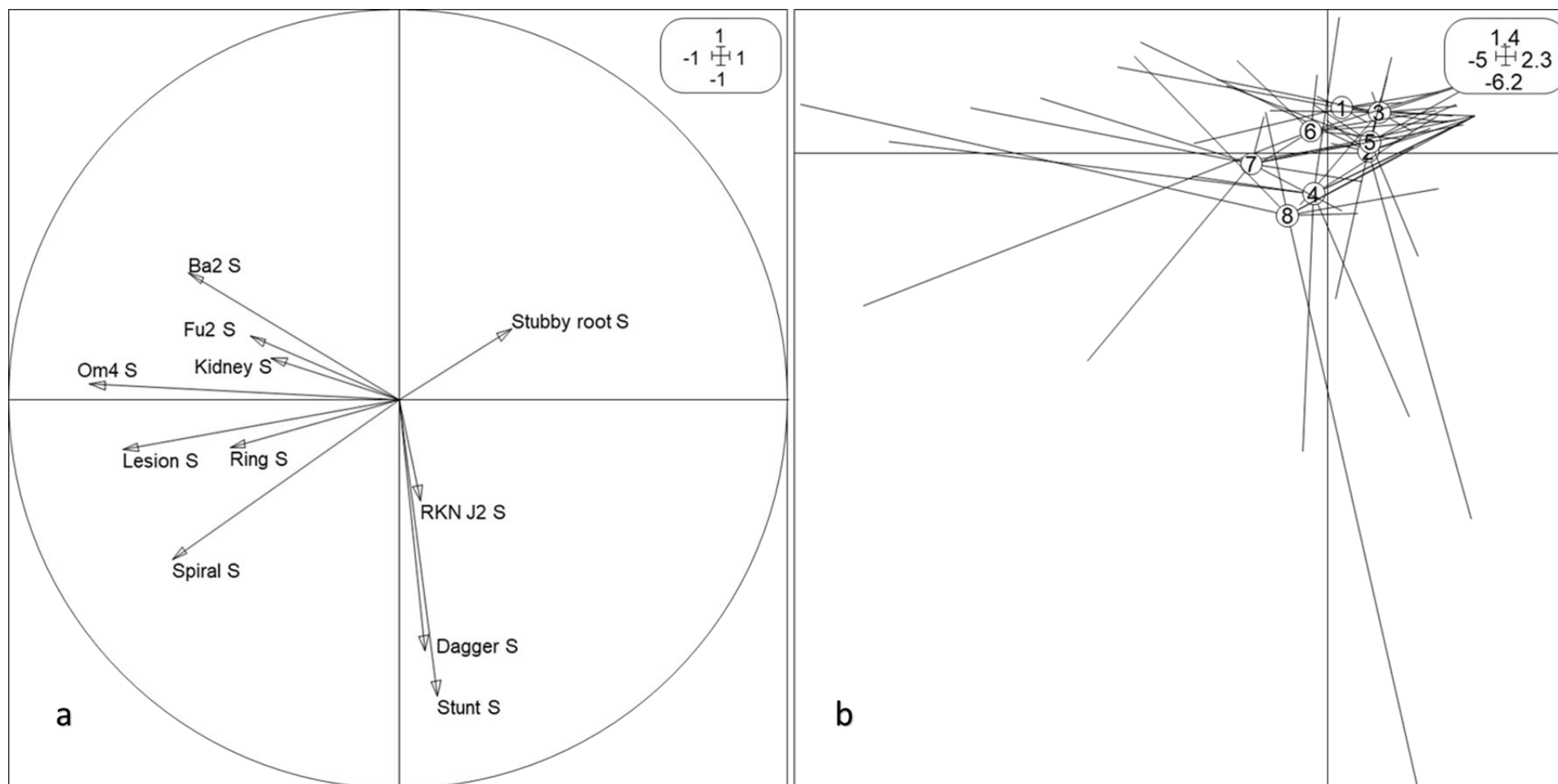


Figure 4.1 a: Pre-plant nematode analysis (sampling interval 1) from soil samples, with b: the relation of these nematode populations to each treatment (R = nematodes in root samples; S = nematodes in soil samples). RKN = root-knot nematodes (*Meloidogyne* spp.); LN = lesion nematodes (*Pratylenchus* spp.); Spiral = spiral nematodes (Hoplolaimidae); Ring = ring nematodes (Criconeematidae); Kidney = Kidney shaped nematode (*Rotylenchulus* spp.); Stubbyroot = stubby root nematodes (Trichodoridae); Dagger = dagger nematodes (*Xiphinema* spp.); Stunt = stunt nematodes (*Tylenchorhynchus* spp.); Ba2 = bacterivore nematodes; Fa2 = fungivore nematodes; Om4 = omnivore nematodes.

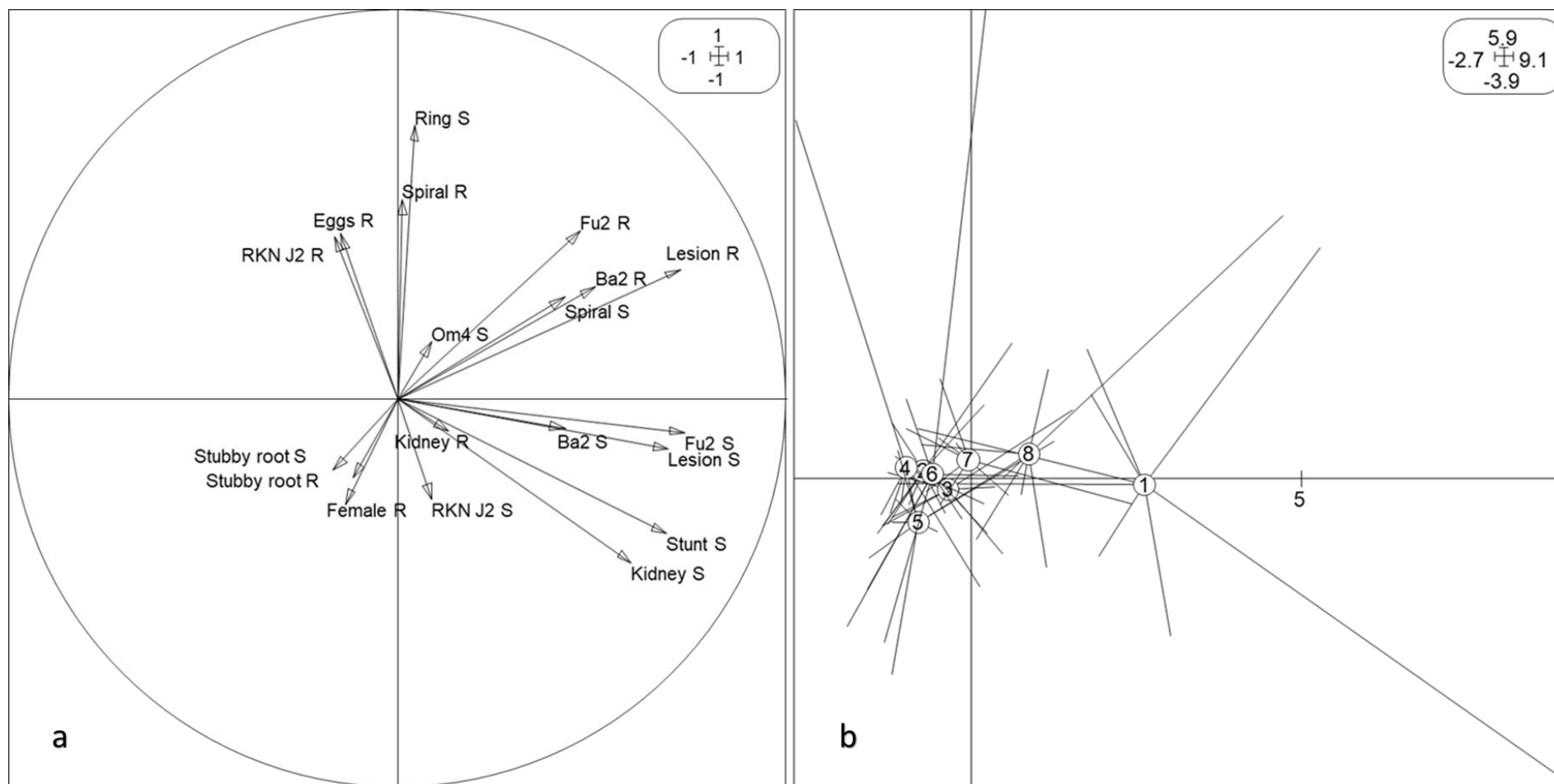


Figure 4.2 a: Nematode analysis at 10 WAE (sampling interval 2) from soil and root samples, with b: the relation of these populations to each treatment (R = nematodes in root samples; S = nematodes in soil samples). RKN/Female = root-knot nematodes (*Meloidogyne* spp.); LN = lesion nematodes (*Pratylenchus* spp.); Spiral = spiral nematodes (Hoplolaimidae); Ring = ring nematodes (Criconeematidae); Kidney = Kidney shaped nematode (*Rotylenchulus* spp.); Stubbyroot = stubby root nematodes (Trichodoridae); Dagger = dagger nematodes (*Xiphinema* spp.); Stunt = stunt nematodes (*Tylenchorhynchus* spp.); Ba2 = bacterivore nematodes; Fa2 = fungivore nematodes; Om4 = omnivore nematodes.

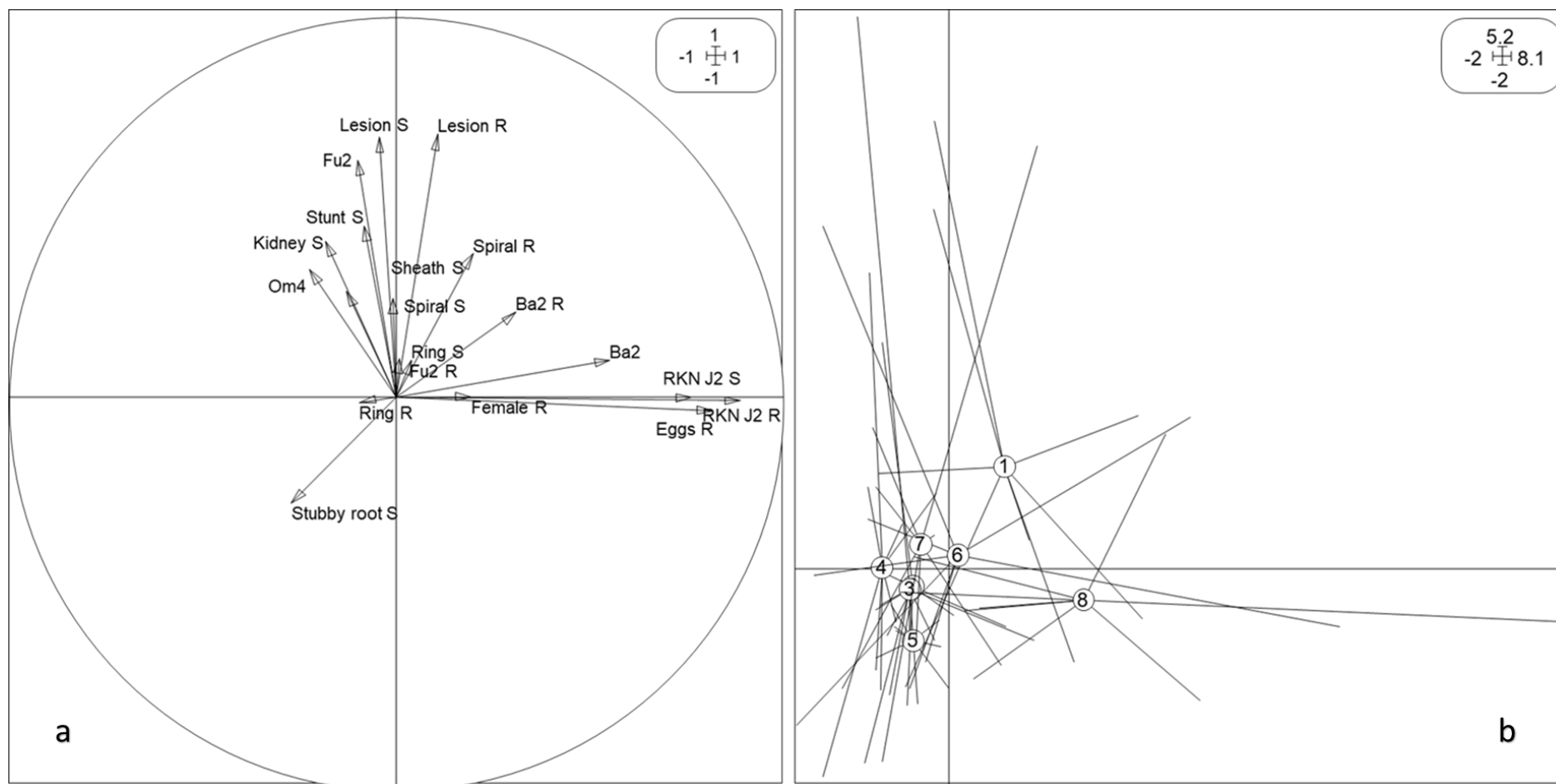


Figure 4.3 a: Nematode analysis at harvest (sampling interval 3) from soil and root samples, with b: the relation of these nematode populations to each treatment (R = nematodes in root samples: S = nematodes in soil samples). RKN/Female = root-knot nematodes (*Meloidogyne* spp.); LN = lesion nematodes (*Pratylenchus* spp.); Spiral = spiral nematodes (Hoplolaimidae); Ring – ring nematodes (Criconematidae); Kidney = Kidney shaped nematode (*Rotylenchulus* spp.); Stubbyroot = stubby root nematodes (Trichodoridae); Dagger = dagger nematodes (*Xiphinema* spp.); Stunt = stunt nematodes (*Tylenchorhynchus* spp.); Ba2 = bacterivore nematodes; Fa2 = fungivore nematodes; Om4 = omnivore nematodes.

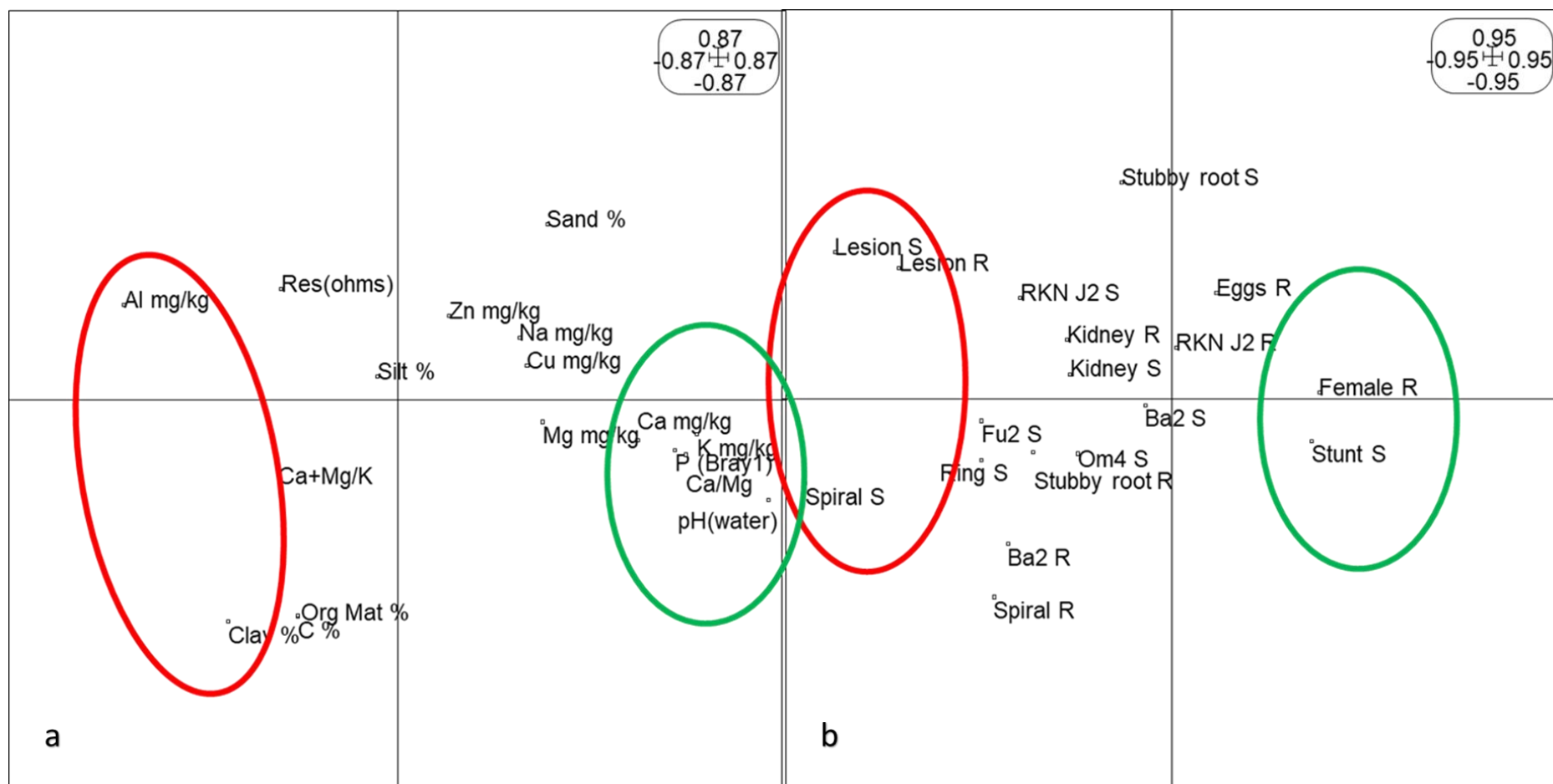


Figure 4.4: Coinertia analysis between a: soil chemo-physical properties and b: log transformed nematode data in soil and roots at 10 WAE (R = nematodes in root samples: S = nematodes in soil samples). RKN/Female = root-knot nematodes (*Meloidogyne* spp.); LN = lesion nematodes (*Pratylenchus* spp.); Spiral = spiral nematodes (Hoplolaimidae); Ring – ring nematodes (Criconematidae); Kidney = Kidney shaped nematode (*Rotylenchulus* spp.); Stubbyroot = stubby root nematodes (Trichodoridae); Dagger = dagger nematodes (*Xiphinema* spp.); Stunt = stunt nematodes (*Tylenchorhynchus* spp.); Ba2 = bacterivore nematodes; Fa2 = fungivore nematodes; Om4 = omnivore nematodes.

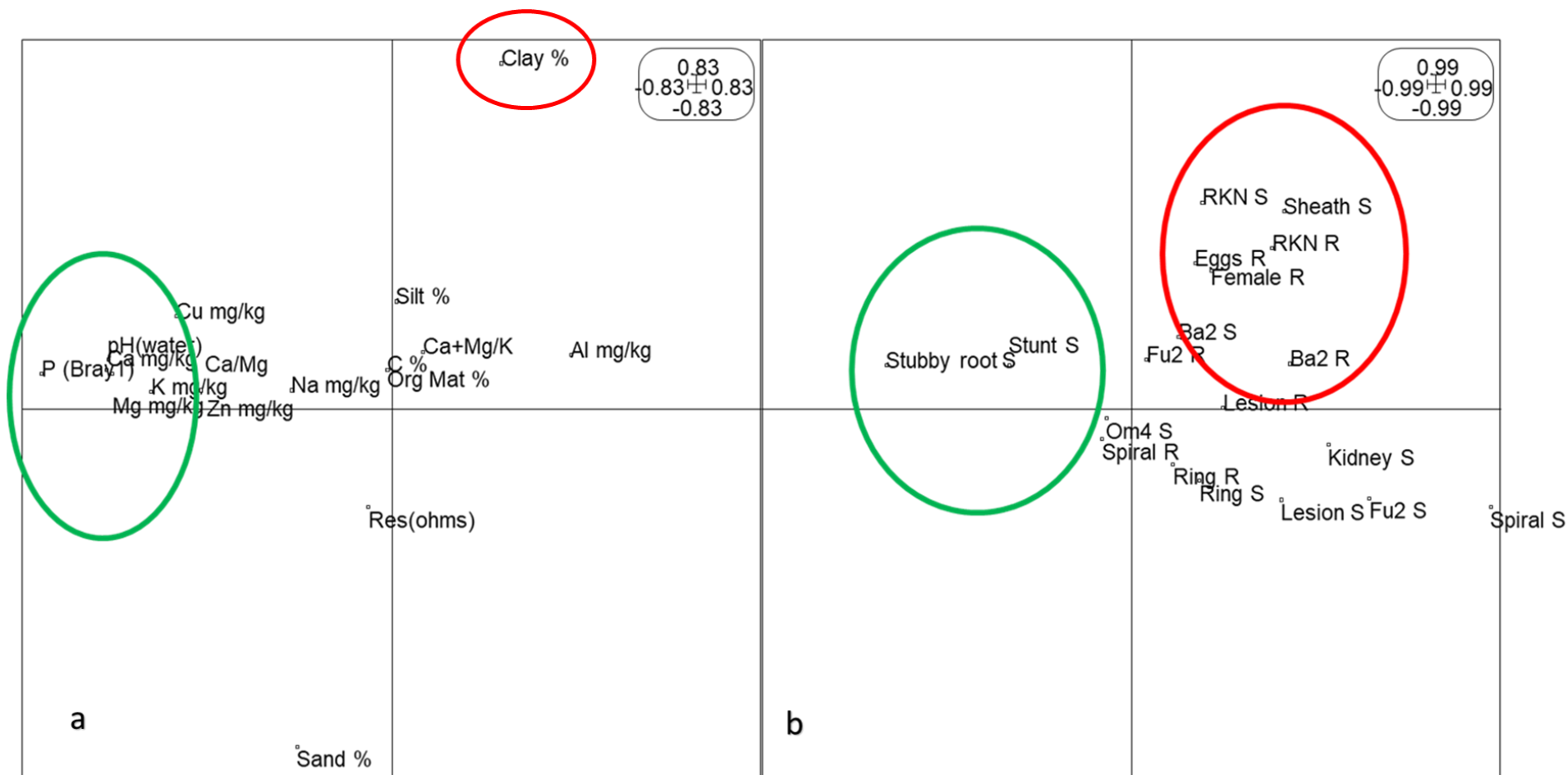


Figure 4.5: Coinertia analysis between a: soil chemo-physical properties and b: log transformed nematode data in soil and roots at harvest (R = nematodes in root samples: S = nematodes in soil samples). RKN/Female = root-knot nematodes (*Meloidogyne* spp.); LN = lesion nematodes (*Pratylenchus* spp.); Spiral = spiral nematodes (Hoplolaimidae); Ring – ring nematodes (Criconeematidae); Kidney = Kidney shaped nematode (*Rotylenchulus* spp.); Stubbyroot = stubby root nematodes (Trichodoridae); Dagger = dagger nematodes (*Xiphinema* spp.); Stunt = stunt nematodes (*Tylenchorhynchus* spp.); Ba2 = bacterivore nematodes; Fa2 = fungivore nematodes; Om4 = omnivore nematodes.

### 4.3.3 Soil faunal analysis (NINJA)

At pre-plant and after fallow sampling intervals, the beneficial and plant-parasitic nematodes were identified and described to family or genus level. A total of 19 families and 20 genera were identified. Table 4.6 lists the different nematodes extracted and identified per trophic group and provides the c-p for each family/genus. Plant-parasitic (herbivore) nematodes extracted were identified from eight families and eight genera, making it the most diverse group of nematodes found in this trial site. Bacterivore nematodes comprised of seven families and eight genera, while fungivore nematodes found only came from three families and four genera. Only one family of omnivore nematodes was extracted and identified in this study.

Table 4.6: Nematode families/genera listed per trophic level, as identified from the various plots in the trial site near Tzaneen (Limpopo province) where this study was conducted. Includes coloniser-persister (c-p) classification as published on Nemaplex (2021).

| Family                | Genus                  | c-p | Family                       | Genus                 | c-p |
|-----------------------|------------------------|-----|------------------------------|-----------------------|-----|
| <b>Herbivores</b>     |                        |     | <b>Bacterivores continue</b> |                       |     |
| <b>Tylenchidae</b>    | <i>Tylenchus</i>       | 2   | Rhabditidae                  |                       | 1   |
| <b>Belondiridae</b>   | <i>Axonchium</i>       | 5   | Monhysteridae                | <i>Monhystera</i>     | 1   |
| <b>Criconematidae</b> |                        | 3   | Plectidae                    | <i>Plectus</i>        | 2   |
| <b>Hoplolaimidae</b>  | <i>Helicotylenchus</i> | 3   | Prismatolaimidae             | <i>Prismatolaimus</i> | 3   |
|                       | <i>Rotylenchulus</i>   | 3   | Alaimidae                    | <i>Alaimus</i>        | 4   |
| <b>Heteroderidae</b>  | <i>Meloidogyne</i>     | 3   | Osstellidae                  |                       | 2   |
| <b>Trichodoridae</b>  | <i>Paratrichodorus</i> | 3   | <b>Fungivores</b>            |                       |     |
| <b>Pratylenchidae</b> | <i>Pratylenchus</i>    | 3   | Aphelenchoididae             | <i>Aphelenchoides</i> | 2   |
| <b>Longidoridae</b>   | <i>Xiphinema</i>       | 5   |                              | <i>Aphelenchus</i>    | 2   |
| <b>Bacterivores</b>   |                        |     | Anguinidae                   | <i>Ditylenchus</i>    | 2   |
| <b>Cephalobidae</b>   | <i>Cephalobus</i>      | 2   | Diphtherophoridae            | <i>Diphtherophora</i> | 3   |
|                       | <i>Eucephalobus</i>    | 2   | <b>Omnivores</b>             |                       |     |
|                       | <i>Acrobeloides</i>    | 2   | Dorylaimidae                 |                       | 4   |
|                       | <i>Acrobeles</i>       | 2   |                              |                       |     |

The total number of nematodes extracted per trophic group at the pre-plant (A) interval and after fallow (B) interval, which occurred 32 months after the pre-plant sampling interval, is shown in Table 4.7. The percentage change in population densities over time is also shown for each treatment.

Table 4.7: Total number of nematodes extracted per treatment at pre-plant (A) interval and after fallow (B) interval, showing the percentage change in population densities over time (positive value indicates population increase, while a negative value indicates a population decline over the 32-month period).

| Treatment           | 1                 | 2                                                 | 3                              | 4                                    | 5                                                   | 6                                                             | 7                                  | 8                                |
|---------------------|-------------------|---------------------------------------------------|--------------------------------|--------------------------------------|-----------------------------------------------------|---------------------------------------------------------------|------------------------------------|----------------------------------|
|                     | Untreated control | Nemacur® 400 EC, Fenamiphos @ 400 g / L, IRAC: 1B | Nemacur® 400 EC + Kalahari 3:1 | Nemacur® 400 EC +Velum® Prime 400 SC | Nemacur® 400 EC +Velum® Prime 400 SC + Kalahari 3:1 | Velum® Prime 400 SC, Fluopyram @ 400 g / L, IRAC: 25; FRAC: 7 | Velum® Prime 400 SC + Kalahari 3:1 | Kalahari 3:1, organic fertiliser |
| Free-living total A | 7600              | 8450                                              | 5520                           | 17000                                | 8700                                                | 8400                                                          | 14850                              | 10600                            |
| Free-living total B | 17900             | 13950                                             | 22300                          | 32500                                | 25100                                               | 18500                                                         | 15700                              | 21600                            |
| % Change            | <b>136</b>        | <b>65</b>                                         | <b>304</b>                     | <b>91</b>                            | <b>189</b>                                          | <b>120</b>                                                    | <b>6</b>                           | <b>104</b>                       |
| Bacterivores A      | 5127              | 7040                                              | 1620                           | 13350                                | 6050                                                | 5250                                                          | 12700                              | 7600                             |
| Bacterivores B      | 14650             | 12300                                             | 18450                          | 25900                                | 18450                                               | 14000                                                         | 10450                              | 16000                            |
| % Change            | <b>186</b>        | <b>75</b>                                         | <b>1039</b>                    | <b>94</b>                            | <b>205</b>                                          | <b>167</b>                                                    | <b>-18</b>                         | <b>111</b>                       |
| Fungivore A         | 2003              | 1410                                              | 3900                           | 1550                                 | 2150                                                | 1800                                                          | 450                                | 2050                             |
| Fungivore B         | 2150              | 1250                                              | 2200                           | 5000                                 | 4450                                                | 2850                                                          | 30260                              | 4850                             |
| % Change            | <b>7</b>          | <b>-11</b>                                        | <b>-44</b>                     | <b>223</b>                           | <b>107</b>                                          | <b>58</b>                                                     | <b>6624</b>                        | <b>137</b>                       |
| Omnivores A         | 470               | 0                                                 | 0                              | 2100                                 | 500                                                 | 1350                                                          | 1700                               | 950                              |
| Omnivores B         | 1100              | 250                                               | 1650                           | 1600                                 | 2200                                                | 1650                                                          | 2200                               | 750                              |
| % Change            | <b>134</b>        | <b>-</b>                                          | <b>-</b>                       | <b>-24</b>                           | <b>340</b>                                          | <b>22</b>                                                     | <b>29</b>                          | <b>-21</b>                       |
| Herbivores A        | 4150              | 5100                                              | 5203                           | 11000                                | 5600                                                | 7950                                                          | 13550                              | 11800                            |
| Herbivores B        | 1150              | 500                                               | 250                            | 4200                                 | 3950                                                | 2800                                                          | 2650                               | 2800                             |
| % Change            | <b>-72</b>        | <b>-90</b>                                        | <b>-95</b>                     | <b>-62</b>                           | <b>-29</b>                                          | <b>-65</b>                                                    | <b>-80</b>                         | <b>-76</b>                       |

The overall composition and change in the nematode assemblage feeding types as a result of the fallow period are shown in Figure 4.6. From this, it is evident that the herbivore component in each treatment decreased from the pre-plant sampling to the after-fallow sampling interval, while the bacterivore component increased between the two sampling times for all treatments. The fallow period resulted in a slight increase in the omnivore component for all treatments. Changes in the fungivore component varied, where considerable changes were only seen in treatments 2 and 3, while densities in the other treatments remained similar between sampling intervals. In terms of predators, which feed on all types of nematodes and protozoa, none were present in any of the soil or root samples taken from the study site.

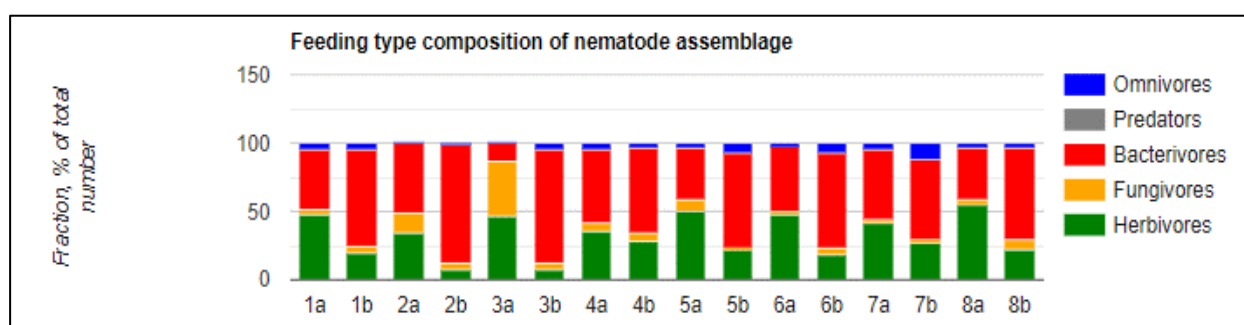


Figure 4.6. Feeding type composition of the total nematode assemblage in the trial site of both sampling intervals. The treatments are marked numerically, while the sampling intervals are indicated with letters (a = pre-plant interval; b = after fallow interval). Treatments represented; 1 = Untreated control; 2 = Nematicur® 400 EC; 3 = Nematicur® 400 EC + Kalahari 3:1; 4 = Nematicur® 400 EC + Velum® Prime 400 SC; 5 = Nematicur® 400 EC + Velum® Prime 400 SC + Kalahari 3:1; 6 = Velum® Prime 400 SC; 7 = Velum® Prime 400 SC + Kalahari 3:1; 8 = Kalahari 3:1.

The overall maturity of the soil was fairly low, as shown by MI data for the treatments and the pre-plant (a) and after fallow (b) sampling intervals in Figure 4.7, with slight variation between treatments and sampling times. This is evident for all treatments except treatments 5, 7 and 8, where the maturity index improved slightly over time.

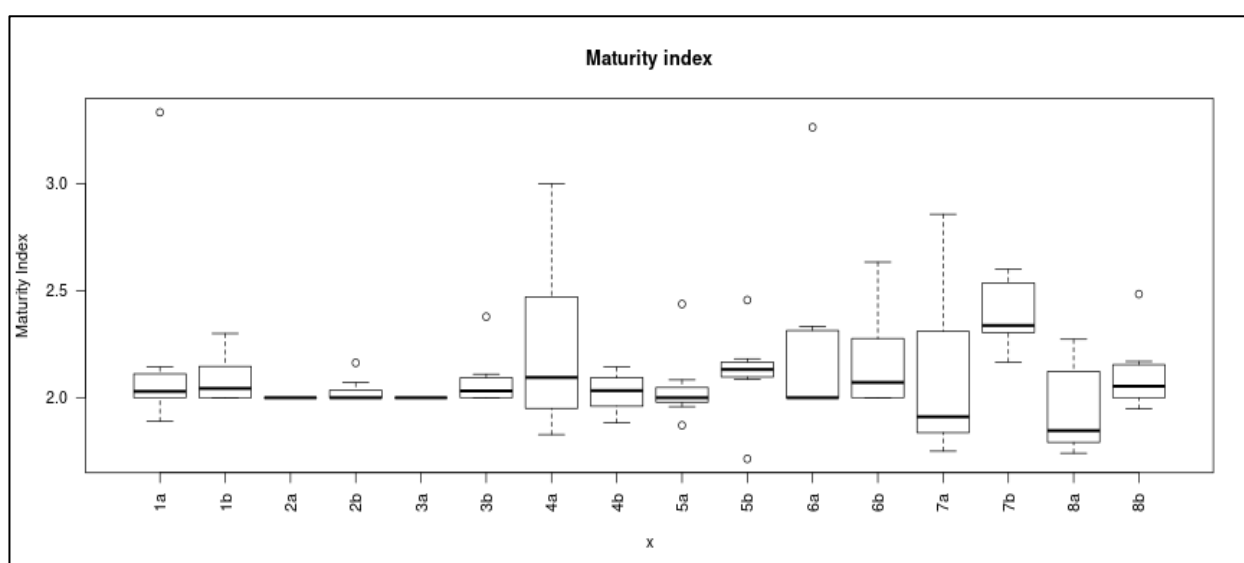


Figure 4.7. Maturity index (MI) of trial site at pre-plant and after fallow period sampling intervals. The treatments are marked numerically, while the sampling intervals are indicated with letters (a = pre-plant interval; b = after fallow interval). Treatments represented; 1 = Untreated control; 2 = Nematicur® 400 EC; 3 = Nematicur® 400 EC + Kalahari 3:1; 4 = Nematicur® 400 EC + Velum® Prime 400 SC; 5 = Nematicur® 400 EC + Velum® Prime 400 SC + Kalahari 3:1; 6 = Velum® Prime 400 SC; 7 = Velum® Prime 400 SC + Kalahari 3:1; 8 = Kalahari 3:1.

The metabolic footprints of the different nematodes identified from each treatment at the pre-plant and after the fallow sampling intervals were used to quantify the overall carbon utilisation of different food web components (Ferris, 2010) as illustrated in Figure 4.8. Most of the treatments are concentrated in the 3<sup>rd</sup> quadrat of the metabolic footprint analysis, with low EI and SI (below 40), except for treatment 8 with an intermediate SI of 50. This quadrat indicated that a soil can be characterised as degraded and depleted with a high carbon to nitrogen ratio, while being mostly populated by fungivores belonging to c-p 2. Only treatment 8, at pre-plant, was situated in a different quadrat, namely quadrat 2, with an intermediate EI between 50 and 60, but low SI (between 20 and 30). Quadrat 2 characterises a soil as disturbed and nitrogen enriched, with a low carbon to nitrogen ratio, mostly populated by bacterivores. When comparing this result to the schematic interpretation of the food web analysis (Chapter 1, Figure 1.12), the data indicated that the trial field was overall degraded and depleted at both sampling times.

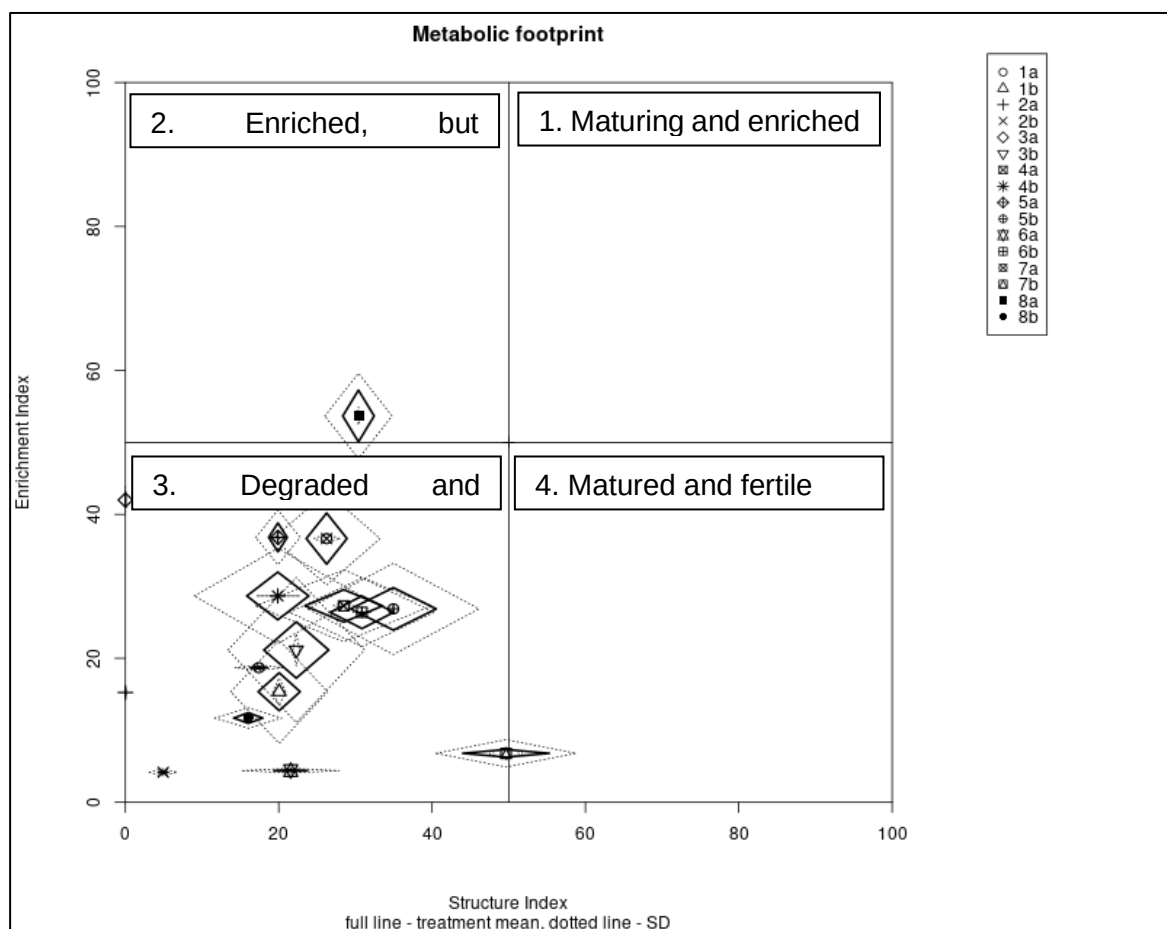


Figure 4.8. Metabolic footprints of beneficial nematode for each treatment at each sample interval. The treatments are marked numerically, while the sampling intervals are indicated with letters (a = pre-plant; b = after fallow period). Treatments represented; 1 = Untreated control; 2 = Nemacur® 400 EC; 3 = Nemacur® 400 EC + Kalahari 3:1; 4 = Nemacur® 400 EC + Velum® Prime 400 SC; 5 = Nemacur® 400 EC + Velum® Prime 400 SC + Kalahari 3:1; 6 = Velum® Prime 400 SC; 7 = Velum® Prime 400 SC + Kalahari 3:1; 8 = Kalahari 3:1

#### 4.4 Discussion

The beneficial nematodes extracted from the soil and root samples taken from the trial site consisted predominantly of bacterivores, from seven families and eight genera. The most diverse family from this trophic group was Cephalobidae Filipjev, 1934, of which four genera were identified viz. *Cephalobus* Bastian, 1865, *Eucephalobus* Steiner, 1936, *Acrobelloides* Cobb 1924 and *Acrobeles* von Linstow, 1877. Fungivore nematodes from three families and four genera were also extracted from the soil and root samples. From the family Aphelenchoididae, 2 genera viz. *Aphelenchoides* Fischer, 1894 and *Aphelenchus* Bastian, 1865 were identified, making this the most diverse family of fungivores extracted from the soil and root samples in this study. These microbial grazing nematodes are critical, as they play a key role in nitrogen and nutrient mineralisation (Ferris *et al.*, 2004; Puissant *et al.*, 2021). Their population densities and diversity are also greatly affected by plant diversity, resource availability and environmental conditions (Ferris *et al.*, 2004; Puissant *et al.*, 2021; Zhu *et al.*, 2018). Only one family of omnivores (Dorylaimidae De Man, 1876) was extracted from the soil samples taken from the study site. This family is known to be extremely sensitive to various agricultural practices and is, therefore, a very good indicator of soil disturbances (Gomes *et al.*, 2003).

Results from this study for the two sampling intervals, pre-plant and after grass fallow (27 months after the harvest sampling interval), showed no significant differences among the treatments for the respective beneficial nematode trophic groups or the total nematode community. Nonetheless, it showed that Kalahari 3:1, especially when used as a stand-alone application, showed a trend that it was advantageous for the beneficial soil nematode communities, in that its abundance generally increased, as compared to that of the chemical nematicide treatments. This was evident since a 104% increase in the total beneficial nematode community was experienced over the 32-month duration between the pre-plant (first) and after fallow sampling intervals, especially for bacterivores which increased by 111%. The lowest and highest increase in total beneficial soil nematodes for the synthetic nematicides were 120% for Velum® Prime 400 SC and 65% for NemaCur® 400 EC. Although the percentage increase in beneficial soil nematodes for Velum® Prime was higher than that of Kalahari 3:1, the total number of beneficial nematodes after the fallow was higher for Kalahari 3:1 (21600) than for Velum® Prime (18500). In addition, the stand-alone Kalahari 3:1 treatment showed a slight increase in MI, placing it in quadrat 2 (more enriched, but disturbed) according to faunal analysis. This change was however not significantly different from the other treatments but may be indicative of a trend that may proceed over a longer period than measured in this study. These trends correlate with the findings of Lazarova *et al.* (2021), where they showed that bacterivore nematode numbers tend to increase in response to an increase in organic matter in apple and banana (*Musa* L.). The total number of beneficial nematodes varied over time in treated and untreated plots in the present study, which

correlates with the results obtained by Gruzdeva *et al.* (2007), who found that changes in the nematode community composition, as well as population densities of different genera, depended on the type of fertilisers applied in a long-term study on a meadow planted with timothy grass (*Phleum pratense* L.). In a long-term biodiversity study conducted by Dietrich *et al.* (2021) in Germany, focusing on nine different plant species, including five different types of grass, it was found that two grass species *viz.* bulbous oat grass (*Arhenatherum elatius* (L.) P.Beauv. ex J.Presl & C.Presl, 1819) and Cock's-foot grass (*Dactylis glomerata* L.), had an overall positive effect on nematode diversity. Their study further found that nematode communities were greatly impacted by plant species diversity. Bacterivores especially were more diverse and abundant in plots with more plant species mixtures than in monocropped plots. Their study also showed that PPN abundance was higher in monocropped plots than in grass and mixed plant species plots. This supports the findings in the present study, where bacterivore abundance increased, while PPN abundance decreased slightly after the 27-month White Buffalo grass fallow period. Regarding *M. javanica*, the predominant PPN species that occurred in the Deerpark trial site where this project was done in particular, this correlates with a report by Lenné (1981) that White Buffalo grass is a poor host to this RKN species in Brazil.

From the results of the present study, it would seem that bacterivore nematodes were more resilient against environmental changes and increased with the increase of available organic matter as shown in the multivariate PCA results. No information in this regard could be found for potato studies, but this trend partially coincides with the findings of Liu *et al.* (2016), where the addition of amendments (straw and mineral fertilisers) showed an increase both in bacterivore (*Eucephalobus*) and fungivore (*Filenchus*) nematode population densities in a rice (*Oryza sativa* L.)-wheat (*Triticum aestivum* L.) agroecosystem. The results from this study showed an increase in only bacterivore nematode abundance similar to that observed in the study conducted by Liu *et al.* (2016), while fungivore nematode abundance did not correlate with the increase in organic material. The study of Herren *et al.* (2020) also did not find a correlation between increased organic material and fungivore nematode abundance and supports the findings of this study. The article stated that compost effects on soil nematodes in various crops can be variable and this is supported by various other studies (Herren *et al.*, 2020; Renčo *et al.*, 2010; Steel *et al.*, 2012; Thoden *et al.*, 2011). The effects of neem-amendments have not been as widely studied as the effect of composts, however, a study by Abbasi *et al.* (2005) found that the addition of different neem seed-cake treatments to soil increased or had no significant effect on the beneficial soil nematode population densities in a tomato (*Solanum lycopersicum* L.) study in Canada, which correlates with what was observed in this study. Another study by Langat *et al.* (2008), however, contradicts this, as their findings showed that neem significantly reduced beneficial soil

nematodes to a level comparable to that obtained with fenamiphos (Nemacur®) in carnation (*Dianthus caryophyllus* L.) in Kenya.

Coinertia analysis identified correlations between soil chemical parameters and nematode communities. However, no obvious correlations with specific parameters were apparent. At 10 WAE, pH and Ca (mg/kg) were correlated with elevated levels of RKN in the roots and stubby root nematodes in soil and roots while at harvest, RKN was positively related with Al (mg/kg) and % clay. This corroborates the findings by Nielsen *et al.* (2014), who sampled 12 environmentally diverse regions globally and found that nematode family abundance and diversity showed no significant correlations with local environmental properties. A more recent study by Nguyen *et al.* (2020) found that the abundance of PPN was closely related to soil pH, while beneficial soil nematodes were related to the soil electrical conductivity (EC). These kinds of correlations were not observed in this trial.

The effect of a natural fallow period, with the natural growth of White Buffalo grass, did not seem to improve the soil health nor did it significantly impact on the overall beneficial soil nematode community composition over time. However, PPN abundance decreased (by 72%), substantiating results by Lenné (1981) specific for *M. javanica* that this grass is a poor host of this RKN species, while bacterivore abundance increased (by 186%) over time in the untreated plots (purely the effect of the grass fallow). Adediran *et al.* (2005) showed that PPN population densities were suppressed by 30-49% with natural bush regrowth fallow over 12, 18, 24 and 30 months in different agroecological systems in south-western Nigeria, which supports the findings in this trial. The natural bush regrowth fallow showed the lowest percentage suppression of PPN than other cover crops tested in the trial by Adediran *et al.* (2005). The findings in this trial were also supported by that of Nguyen *et al.* (2020), who showed that, although the abundance of different nematode trophic groups changes in the Mekong Delta in Vietnam during seasons, the overall nematode community composition remained similar over three climatic cultivation seasons between 2017 and 2018, in a multi-year rice cropping system.

During the extreme drought conditions experienced under high temperature conditions from the onset of this trial, 627 mm rainfall experienced in the 2018/2019 season and 744 mm in the 2019/2020 season compared to an average of 1148 mm during previous years and the subsequent stress to the crop are suggested to have played an extensive role in the inconclusive nematode data obtained and poor crop yield. The drought started in the 2014/2015 season, where only 443 mm rainfall was experienced in this area (38% of the average rainfall from before the onset of drought in this area). Persisting drought conditions could be a result of climate change and pose a great challenge to agricultural production and research such as this study. Climate change is known to result in irregular weather patterns, drought, extreme fluctuations in

precipitation and temperature (Rapp, 2014), all of which were experienced during the duration of this trial. However, there is very little research available regarding the long-term effects of climate change on beneficial soil nematodes and in this respect, the present study represents some baseline data.

Besides the extreme drought conditions experienced during the duration of this study, the length of the grass fallow period may have been too short to result in substantial changes in the beneficial nematode community. The lack of plant species variation and abundance during the fallow period may also have played a role. The study conducted by Dietrich *et al.* 2021 showed that over 15 years, PPN abundance was higher in monocrop (single fallow plant species) treatments than in treatments that contained a mixture of plants, including various grasses. Moreover, the potentially insufficient content of both neem and manure in the Kalahari 3:1 product could also have contributed to the lack of impact experienced on the beneficial community. This was mentioned as a potential contributing factor to the lack of reduction in PPN densities in Chapter 3, paragraph 3.4.

The insignificant change in MI over time would suggest that just leaving a field after cultivation would not necessarily increase the overall soil health and additional management practices are needed to improve this specific field's health/fertility. This suggests that the soil was stressed before planting activities commenced and very little changed over time. The c-p triangle indicated that the soil was and remained stressed over time. This is supported by the minimal changes in omnivore abundance in the field over time, indicating that the soil remained disturbed from before the trial was initiated and that this did not improve during the fallow period. As stated by Gomes *et al.* (2003), omnivores from the Dorylaimidae, which was the only family of omnivores found in both the soil and root samples taken from the field, are very sensitive to soil disturbances caused by agricultural practices.

#### **4.5 Conclusion**

From the results obtained, it can be concluded that the stand-alone neem-amended cow manure treatment (8), Kalahari 3:1, had the most beneficial effect (although not significant) on the nematode population composition and abundance, compared to standard nematicides and combinations of these standards with Kalahari 3:1. There did not seem to be any significant associations between soil properties and nematode population abundance and diversity in this trial. Neither the standard nematicides, nor the organic treatment seemed to influence the overall soil health and additional actions will be needed to improve the overall soil health.

More research over a longer period is needed to conclusively prove the beneficial effect of the Kalahari 3:1 product on beneficial nematodes, preferably during more favourable growing

conditions. Further studies could also be conducted to determine which additional agricultural management practices would improve the soil health of a site such as the one used in this study, while also improving the beneficial soil nematode community and promoting more sustainable, environmentally friendly agriculture. Improvement of the Kalahari 3:1 product to contain higher concentrations of neem and manure may also benefit soil health, while conducting this type of research under more favourable environmental conditions is also suggested.

Ultimately, the use of White Buffalo grass may be a potential strategy to be considered in an integrated pest management approach for use in local potato fields to reduce *M. javanica* population densities as was shown in Brazil by Lenné (1981). Dedicated host status screenings for this grass against all economically important RKN species must however to be done to confirm this.

#### 4.6 References

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## Chapter 5: Conclusion and future prospects

### 5.1 Testing of the hypotheses

This study was aimed at providing farmers and the potato industry with integration of more sustainable, eco-friendly management options to reduce plant-parasitic nematode (PPN) population densities and the subsequent damage to crops these organisms cause, while improving soil health. This study was prompted by the increasing pressure on farmers and the crop protection industry by governing and ecological groups to reduce the use of hazardous substances to control pests and diseases, such as PPN in potato (*Solanum tuberosum* L.). Potato is considered a crucial crop in the scope of food security for a rapidly growing global human population (Stokstad, 2019). The study compared two registered chemical nematicides, in different danger classes (Nemacur 400 EC and Velum Prime 400 SC), to an organic fertilizer amended with neem (*Azadirachta indica* A. Juss.) oil as single products and in different combinations. Selected soil properties were also determined, as these could affect the biological processes in the soil and cannot be ignored when studying soil health (Munoz-Rojas *et al.*, 2018). The effect of the products applied, as well as the effect of the natural White Buffalo grass (*Urochloa mosambicensis* (Hack.) Dandy.) fallow period, on the beneficial soil nematodes were also studied, as these terrestrial nematodes are used as soil health bio-indicators (Habig *et al.*, 2018). The hypotheses were investigated by completing the objectives of this study.

The hypothesis formulated at the beginning of this study was: *‘The use of an easy to use, granulated cow manure amended with neem oil (Kalahari 3:1) is expected to offer nematicidal activity, comparable to that of registered nematicides, against PPN infecting potato under typical South African field conditions, while increasing tuber quality. Use of Kalahari 3:1 succeeded by a natural grass fallow are also foreseen to improve soil health resulting in higher abundance and more diverse beneficial soil nematode communities compared to nematicide-treated areas.’*

Although no information exists for nematode management in potato fields on the specific use of a similar neem-amended manure granule as used in this study, the use of neem products (leaves, oil cakes, oil) and animal manures, either alone or in combination, have previously shown to be promising alternatives for use against PPN populations on various crops to reduce the population densities and damage caused by various PPN species (Osei *et al.*, 2017). The findings of this study did not entirely correlate with these studies. Firstly, due to severe drought and heat experienced in the study timeline, crop emergence was poor, and yields were very low for all treatments. From the few tubers that were harvested, it was clear that using the neem-amended cow manure Kalahari 3:1 on its own would not be economical. This treatment had the lowest number of tubers harvested compared to the other treatments used, including the untreated

control. When combined with one or both registered nematicides, the number of tubers that could be harvested was higher than when the products were used as standalone products.

In terms of controlling root-knot nematodes (RKN) and other PPN populations, the Kalahari 3:1 product did not perform as expected, as did the registered nematicides, and the results for controlling RKN populations and damage in this study contradict those from previous studies where other neem-based plant parts/products (e.g., oil) were used. The treatment that offered the best RKN control was the combination of Nemacur 400 EC with Velum Prime 400 SC. In this regard, the study disproves the stated hypothesis. An interesting observation was that the results from this study, with regards to lesion nematode (LN) population densities, showed that the effect of the neem-amended cow manure followed the trend described by previous studies although results were not statistically significant.

From previous studies, the use of neem leaves and neem seed oil cakes seemed to have good nematicidal effects against various PPN (Campos *et al.*, 2016; Jones, 2017; Khalil, 2013). The authors also describe that the manures used were raw, composted or made into compost tea and were not granulated. This could mean that in the manufacturing process of the Kalahari 3:1 product, the concentration of neem in cow manure is somewhat reduced or too low before processing. Another factor to consider is the prevailing drought conditions that could have played a role in the results obtained.

In conclusion, the use of a nematicide was essential in this specific field to reduce PPN population densities and in this study, the product offering the best nematicidal potential was Nemacur 400 SC; however, the effect was not optimal as was expected and reported (Sesweni, 2016). The organic Kalahari 3:1 product amended with neem oil did not offer the level of nematicidal potential as described in previous research (Jumaah, 2015; Singh & Hali, 2017; Wendimu, 2021), but the product did seem to slightly improve the nematicidal effect of the registered nematicides when combined in the field.

In terms of beneficial soil nematode data recorded in this study, the hypothesis could also not be supported. Findings published by Gruzdeva *et al.* (2007) and Liu *et al.* (2016) indicated that the addition of organic manures would increase the population densities of bacterivore and fungivore nematodes in the soil. Although the results from this study showed that the total number of beneficial soil nematodes varied over time in all treated and untreated plots, it to a certain degree supports that of the mentioned authors. Bacterivore nematode populations seemed to be more resilient in this South African field with its abundance increasing more in plots treated with the Kalahari 3:1 product than in plots left untreated or treated with only the chemical nematicides.

Fungivore populations however, seemed to be more sensitive to agricultural practices and treatments than the populations studied by Liu *et al.* (2016). This varied response of beneficial soil nematode populations to the addition of organic compost is supported by other studies (Herren *et al.*, 2020). The results obtained from this trial proves the stated hypothesis. The use of the neem-amended cow manure as a standalone product had the most beneficial effect on the overall beneficial soil nematode population diversity and density, in comparison to the use of registered nematicides, whether used singly or in the various combinations tested. In this trial, there were no significant associations between soil properties and beneficial soil nematode community spread relating to results found by Nielsen *et al.*, 2014.

When comparing the effects of the natural White Buffalo grass fallow period on the composition of soil nematodes in this field to the soil nematode community of the field before treatments and planting commenced, the fallow period generally increased the population densities of bacterivores while reducing the densities of herbivores. Unfortunately, there was not much change between the MI of the field from pre-plant to after fallow, suggesting that the Kalahari 3:1 and the White Buffalo grass fallow period alone did not improve the overall soil health significantly over the 27 months it was applied. After subjecting the field data to NINJA and studying the metabolic footprint analysis, the soil nematode populations were concentrated mostly in the 3<sup>rd</sup> quadrat; thus, the soil is classified as degraded and depleted. To improve the overall soil health and beneficial soil nematode community composition, additional management and improvement practices would thus be needed.

## **5.2 Potential future research**

From various articles, the nematicidal potential of neem and animal manure has repeatedly been shown and could reduce the number of chemicals required to control PPN populations. The results obtained from this study contradicts these findings, but this could be due to the extreme drought conditions experienced, too low concentrations of neem in the manure granules, the overall formulation of the product, or the recommended dosage could have been too low for this specific field. Further research could include conducting this research under more favourable environmental conditions as well as using different and/or higher concentrations of neem oil in the cow manure granules. Another option would be to use the neem oil concentration tested in this trial in an ungranulated, powder formulation of cow manure to investigate if the formulation could have reduced the product efficacy.

Fluopyram (Velum Prime 400 SC), which was the less hazardous registered nematicide used in this trial should be tested against high populations of LN in soil (any crop) to potentially elevate the status of this product to become a more effective tool for farmers to utilize against this genus

of PPN. A new active ingredient named fluazaindolizine shows good potential for PPN control in China and could be compared against fluopyram and fenamiphos in South African, sandy soil conditions for potato farmers.

More research on the PPN host status of endemic South African grass species, that can be used by local potato producers, to economically important RKN and LN species is needed, while also evaluating the potential of these grasses as cover crops/crop rotations.

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